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(54) Title: <b>Hu-B1.219, A NOVEL HUMAN HEMATOPOIETIN RECEPTOR</b>			
(57) Abstract <p>The present invention relates to a novel member of the hematopoietin receptor family, herein referred to as Hu-B1.219. In particular, the invention relates to nucleotide sequences and expression vectors encoding Hu-B1.219 gene product. Genetically engineered host cells that express the Hu-B1.219 coding sequence may be used to evaluate and screen for ligands or drugs involved in Hu-B1.219 interaction and regulation. Since Hu-B1.219 expression has been detected in certain human fetal tissues and cancer cells, molecular probes designed from its nucleotide sequence may be useful for prenatal testing and cancer diagnosis.</p>			
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## Hu-B1.219, A NOVEL HUMAN HEMATOPOIETIN RECEPTOR

### 1. INTRODUCTION

The present invention relates to a novel member of the  
5 hematopoietin receptor family, herein referred to as  
Hu-B1.219. In particular, the invention relates to  
nucleotide sequences and expression vectors encoding Hu-  
B1.219 gene product. Genetically engineered host cells that  
express the Hu-B1.219 coding sequence may be used to evaluate  
10 and screen for ligands or drugs involved in Hu-B1.219  
interaction and regulation. Since Hu-B1.219 expression has  
been detected in certain human fetal tissues and cancer  
cells, molecular probes designed from its nucleotide sequence  
may be useful for prenatal testing and cancer diagnosis.

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### 2. BACKGROUND OF THE INVENTION

A variety of diseases, including malignancy and  
immunodeficiency, are related to malfunction within the  
lympho-hematopoietic system. Some of these conditions could  
20 be alleviated and/or cured by repopulating the hematopoietic  
system with progenitor cells, which when triggered to  
differentiate would overcome the patient's deficiency.  
Therefore, the ability to initiate and regulate hematopoiesis  
is of great importance (McCune et al., 1988, Science  
25 241:1632).

The process of blood cell formation, by which a small  
number of self-renewing stem cells give rise to lineage  
specific progenitor cells that subsequently undergo  
proliferation and differentiation to produce the mature  
30 circulating blood cells has been shown to be at least in part  
regulated by specific hormones. These hormones are  
collectively known as hematopoietic growth factors or  
cytokines (Metcalf, 1985, Science 229:16; Dexter, 1987, J.  
Cell Sci. 88:1; Golde and Gasson, 1988, Scientific American,  
35 July:62; Tabbara and Robinson, 1991, Anti-Cancer Res. 11:81;  
Ogawa, 1989, Environ. Health Persp. 80:199; Dexter, 1989, Br.  
Med. Bull. 45:337).

With the advent of recombinant DNA technology, the genes encoding a number of these molecules have now been molecularly cloned and expressed in recombinant form (Souza et al., 1986, Science 232:61; Gough et al., 1984, Nature 5 309:763; Yokota et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:1070; Kawasaki et al., 1985, Science 230:291). These cytokines have been studied in their structure, biology and even therapeutic potential. Some of the most well characterized factors include erythropoietin (EPO), stem cell 10 factor (SCF), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), and the interleukins (IL-1 to IL-14).

These factors act on different cell types at different 15 stages during blood cell development, and their potential uses in medicine are far-reaching which include blood transfusions, bone marrow transplantation, correcting immunosuppressive disorders, cancer therapy, wound healing, and activation of the immune response. (Golde and Gasson, 20 1988, Scientific American, July:62).

Apart from inducing proliferation and differentiation of hematopoietic progenitor cells, such cytokines have also been shown to activate a number of functions of mature blood cells (Stanley et al., 1976, J. Exp. Med. 143:631; Schrader et al., 25 1981, Proc. Natl. Acad. Sci. U.S.A. 78:323; Moore et al., 1980, J. Immunol. 125:1302; Kurland et al., 1979, Proc. Natl. Acad. Sci. U.S.A. 76:2326; Handman and Burgess, 1979, J. Immunol. 122:1134; Vadas et al., 1983, Blood 61:1232; Vadas et al., 1983, J. Immunol. 130:795), including influencing the 30 migration of mature hematopoietic cells (Weibart et al., 1986, J. Immunol. 137:3584).

Cytokines exert their effects on target cells by binding to specific cell surface receptors. A number of cytokine receptors have been identified and the genes encoding them 35 molecularly cloned. Several cytokine receptors have recently been classified into a hematopoietin receptor (HR) superfamily. The grouping of these receptors was based on

the conservation of key amino acid motifs in the extracellular domains (Bazan, 1990, Immunology Today 11:350) (Figure 1). The HR family is defined by three conserved motifs in the extracellular domain of these receptors. The first is a Trp-Ser-X-Trp-Ser (WSXWS box) motif which is highly conserved and located amino-terminal to the transmembrane domain. Most members of the HR family contain this motif. The second consists of four conserved cysteine residues located in the amino-terminal half of the extracellular region. The third is a conserved fibronectin Type III (FN III) domain which is located between the WSXWS box and the cysteines. The members of the HR family include receptors for ligands such as erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF) (Fukunaga, 1990, Cell 61:341), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3), IL-4, IL-5, IL-6, IL-7, and IL-2 ( $\beta$ -subunit) (Cosman, 1990, TIBS 15:265).

Ligands for the HR are critically involved in the maturation and differentiation of blood cells. For example, IL-3 promotes the proliferation of early multilineage pluripotent stem cells, and synergizes with EPO to produce red cells. IL-6 and IL-3 synergize to induce proliferation of early hematopoietic precursors. GM-CSF has been shown to induce the proliferation of granulocytes as well as increase macrophage function. IL-7 is a bone marrow-derived cytokine that plays a role in producing immature T and B lymphocytes. IL-4 induces proliferation of antigen-primed B cells and antigen-specific T cells. Thus, members of this receptor superfamily are involved in the regulation of the hematopoietic system.

### 3. SUMMARY OF THE INVENTION

The present invention relates to a novel member of the HR family, referred to as Hu-B1.219. In particular, it relates to the nucleotide sequences, expression vectors, host cells expressing the Hu-B1.219 gene, and proteins encoded by the sequences.

The invention is based, in part, upon Applicants' discovery of a cDNA clone, Hu-B1.219, isolated from a human fetal liver cDNA library. While the nucleotide sequence of this clone shares certain homology with other HR genes, it is also unique in its structure. Three forms of Hu-B1.219 have been identified, and they differ in sequence only at their 3' ends. The sequences are expressed in certain human fetal and tumor cells. Therefore, a wide variety of uses are encompassed by the present invention, including but not limited to, the diagnosis of cancer, the marking of fetal tissues, and the screening of ligands and compounds that bind the receptor molecule encoded by Hu-B1.219.

For the purpose of the present invention, the designation Hu-B1.219 refers to the complete cDNA sequence disclosed in Figure 2A-2G. In addition, Hu-B1.219 also refers to the partial coding sequences within the cDNA sequence of Figure 2A-2G.

#### 4. BRIEF DESCRIPTION OF THE DRAWINGS

- 20      Figure 1.      A schematic drawing of conserved regions shared by members of HR family.
- Figure 2A-2G.      Nucleotide sequence and deduced amino acid sequence of Hu-B1.219.
- Figure 3A.      Comparison of 3' end nucleotide sequences of the three forms of the Hu-B1.219.
- 25      Figure 3B.      Comparison of 3' end amino acid sequences of the three forms of Hu-B1.219. The \* symbol indicates a stop codon.
- Figure 4.      Comparison of the spacing of conserved amino acids in the FN III domain between
- 30      Figure 5.      Comparison of conserved motifs between HR molecules and Hu-B1.219 in "Block 3".
- Figure 6.      Comparison of conserved motifs between HR molecules and Hu-B1.219 in "Block 6".
- 35

## 5. DETAILED DESCRIPTION OF THE INVENTION

### 5.1. THE Hu-B1.219 CODING SEQUENCE

The present invention relates to nucleic acid and amino acid sequences of a novel member of the HR family. In a specific embodiment by way of example in Section 6, *infra*, a new member of this HR family of receptors was cloned and characterized. The nucleotide coding sequence and deduced amino acid sequence of the novel receptor are unique, and the receptor is referred to as Hu-B1.219. In accordance with the invention, any nucleotide sequence which encodes the amino acid sequence of the Hu-B1.219 gene product can be used to generate recombinant molecules which direct the expression of Hu-B1.219 gene.

Analysis of the Hu-B1.219 sequence revealed significant homology to the FN III domain of the HR family indicating that it was a member of the HR family of receptors. The shared homology between Hu-B1.219 and other known members of the HR family is discussed in Section 6.2, *infra*. However, this receptor also contains regions of previously unreported unique nucleotide sequences.

Northern blot hybridization analysis, indicates that Hu-B1.219 mRNA is highly expressed in cells of hematopoietic origin. In addition, the Hu-B1.219 sequence is expressed in certain tumor cells.

In order to clone the full length cDNA sequence encoding the entire Hu-B1.219 cDNA or to clone variant forms of the molecule, labeled DNA probes made from nucleic acid fragments corresponding to any portion of the partial cDNA disclosed herein may be used to screen the human fetal liver cDNA library. More specifically, oligonucleotides corresponding to either the 5' or 3' terminus of the partial cDNA sequence may be used to obtain longer nucleotide sequences. Briefly, the library may be plated out to yield a maximum of 30,000 pfu for each 150 mm plate. Approximately 40 plates may be screened. The plates are incubated at 37°C until the plaques reach a diameter of 0.25 mm or are just beginning to make contact with one another (3-8 hours). Nylon filters are

placed onto the soft top agarose and after 60 seconds, the filters are peeled off and floated on a DNA denaturing solution consisting of 0.4N sodium hydroxide. The filters are then immersed in neutralizing solution consisting of 1M Tris HCL, pH 7.5, before being allowed to air dry. The filters are prehybridized in casein hybridization buffer containing 10% dextran sulfate, 0.5M NaCl, 50mM Tris HCL, pH 7.5, 0.1% sodium pyrophosphate, 1% casein, 1% SDS, and denatured salmon sperm DNA at 0.5 mg/ml for 6 hours at 60°C.

10 The radiolabeled probe is then denatured by heating to 95°C for 2 minutes and then added to the prehybridization solution containing the filters. The filters are hybridized at 60°C for 16 hours. The filters are then washed in 1X wash mix (10X wash mix contains 3M NaCl, 0.6M Tris base, and 0.02M

15 EDTA) twice for 5 minutes each at room temperature, then in 1X wash mix containing 1% SDS at 60°C for 30 minutes, and finally in 0.3X wash mix containing 0.1% SDS at 60°C for 30 minutes. The filters are then air dried and exposed to x-ray film for autoradiography. After developing, the film is

20 aligned with the filters to select a positive plaque. If a single, isolated positive plaque cannot be obtained, the agar plug containing the plaques will be removed and placed in lambda dilution buffer containing 0.1M NaCl, 0.01M magnesium sulfate, 0.035M Tris HCl, pH 7.5, 0.01% gelatin. The phage

25 may then be replated and rescreened to obtain single, well isolated positive plaques. Positive plaques may be isolated and the cDNA clones sequenced using primers based on the known cDNA sequence. This step may be repeated until a full length cDNA is obtained.

30 It may be necessary to screen multiple cDNA libraries from different tissues to obtain a full length cDNA. In the event that it is difficult to identify cDNA clones encoding the complete 5' terminal coding region, an often encountered situation in cDNA cloning, the RACE (Rapid Amplification of

35 cDNA Ends) technique may be used. RACE is a proven PCR-based strategy for amplifying the 5' end of incomplete cDNAs. 5'-RACE-Ready cDNA synthesized from human fetal liver containing



a unique anchor sequence is commercially available (Clontech). To obtain the 5' end of the cDNA, PCR is carried out on 5'-RACE-Ready cDNA using the provided anchor primer and the 3' primer. A secondary PCR reaction is then carried out using the anchored primer and a nested 3' primer according to the manufacturer's instructions. Once obtained, the full length cDNA sequence may be translated into amino acid sequence and examined for certain landmarks such as a continuous open reading frame flanked by translation initiation and termination sites, a potential signal sequence and transmembrane domain, and finally overall structural similarity to known HR genes.

#### 5.2. EXPRESSION OF Hu-B1.219 SEQUENCE

In accordance with the invention, Hu-B1.219 polynucleotide sequence which encodes the Hu-B1.219 protein, peptide fragments of Hu-B1.219, Hu-B1.219 fusion proteins or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of Hu-B1.219 protein, Hu-B1.219 peptide fragment, fusion proteins or a functional equivalent thereof, in appropriate host cells. Such Hu-B1.219 polynucleotide sequences, as well as other polynucleotides which selectively hybridize to at least a part of such Hu-B1.219 polynucleotides or their complements, may also be used in nucleic acid hybridization assays, Southern and Northern blot analyses, etc.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used in the practice of the invention for the cloning and expression of the Hu-B1.219 protein. Such DNA sequences include those which are capable of hybridizing to the human Hu-B1.219 sequences under stringent conditions. The phrase "stringent conditions" as used herein refers to those hybridizing conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.; (2) employ during hybridization a denaturing agent such as formamide, for

example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Altered DNA sequences which may be used in accordance with the invention include deletions, additions or substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product itself may contain deletions, additions or substitutions of amino acid residues within a Hu-B1.219 sequence, which result in a silent change thus producing a functionally equivalent Hu-B1.219 protein. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine, histidine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: glycine, asparagine, glutamine, serine, threonine, tyrosine; and amino acids with nonpolar head groups include alanine, valine, isoleucine, leucine, phenylalanine, proline, methionine, tryptophan.

The DNA sequences of the invention may be engineered in order to alter an Hu-B1.219 coding sequence for a variety of ends including but not limited to alterations which modify processing and expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc.

In another embodiment of the invention, an Hu-B1.219 or a modified Hu-B1.219 sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors or 5 stimulators of Hu-B1.219 activity, it may be useful to encode a chimeric Hu-B1.219 protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between a Hu-B1.219 sequence and the 10 heterologous protein sequence, so that the Hu-B1.219 may be cleaved away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of a Hu-B1.219 could be synthesized in whole or in part, using chemical methods well known in the art. See, for 15 example, Caruthers et al., 1980, Nuc. Acids Res. Symp. Ser. 7:215-233; Crea and Horn, 1980, Nuc. Acids Res. 9(10):2331; Matteucci and Caruthers, 1980, Tetrahedron Letters 21:719; and Chow and Kempe, 1981, Nuc. Acids Res. 9(12):2807-2817. Alternatively, the protein itself could be produced using 20 chemical methods to synthesize an Hu-B1.219 amino acid sequence in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (e.g., see Creighton, 1983, Proteins 25 Structures And Molecular Principles, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman 30 and Co., N.Y. , pp. 34-49).

In order to express a biologically active Hu-B1.219, the nucleotide sequence coding for Hu-B1.219, or a functional equivalent, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements 35 for the transcription and translation of the inserted coding sequence. The Hu-B1.219 gene products as well as host cells or cell lines transfected or transformed with recombinant Hu-

B1.219 expression vectors can be used for a variety of purposes. These include but are not limited to generating antibodies (i.e., monoclonal or polyclonal) that competitively inhibit activity of an Hu-B1.219 and neutralize its activity; and antibodies that mimic the activity of Hu-B1.219 ligands in stimulating the receptor to transmit an intracellular signal. Anti-Hu-B1.219 antibodies may be used in detecting and quantifying expression of Hu-B1.219 levels in cells and tissues.

10      5.3. EXPRESSION SYSTEMS

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the Hu-B1.219 coding sequence and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y.

A variety of host-expression vector systems may be utilized to express the Hu-B1.219 coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the Hu-B1.219 coding sequence; yeast transformed with recombinant yeast expression vectors containing the Hu-B1.219 coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the Hu-B1.219 coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the Hu-B1.219 coding sequence; or animal cell systems. The expression elements of these systems vary in their strength and specificities. Depending on the

host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage  $\lambda$ , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when cloning in plant cell systems, promoters derived from the genome of plant cells (e.g., heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll  $\alpha/\beta$  binding protein) or from plant viruses (e.g., the 35S RNA promoter of CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used; when generating cell lines that contain multiple copies of the Hu-B1.219 DNA, SV40-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

In bacterial systems a number of expression vectors may be advantageously selected depending upon the use intended for the Hu-B1.219 expressed. For example, when large quantities of Hu-B1.219 are to be produced for the generation of antibodies or to screen peptide libraries, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include but are not limited to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the Hu-B1.219 coding sequence may be ligated into the vector in frame with the lac Z coding region so that a hybrid AS-lac Z protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general,

such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa  
5 protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel et  
10 al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 1987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter,  
15 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast *Saccharomyces*, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II.

20 In cases where plant expression vectors are used, the expression of the Hu-B1.219 coding sequence may be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson et al., 1984, Nature 310:511-514), or the coat protein promoter  
25 of TMV (Takamatsu et al., 1987, EMBO J. 6:307-311) may be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., 1984, EMBO J. 3:1671-1680; Broglie et al., 1984, Science 224:838-843); or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley  
30 et al., 1986, Mol. Cell. Biol. 6:559-565) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques see, for example, Weissbach &  
35 Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp. 421-463; and Grierson &

Corey, 1988, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9.

An alternative expression system which could be used to express Hu-B1.219 is an insect system. In one such system, 5 Autographa californica nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The Hu-B1.219 coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under 10 control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the Hu-B1.219 coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin 15 gene). These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed. (e.g., see Smith et al., 1983, J. Virol. 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral based 20 expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the Hu-B1.219 coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene 25 may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing Hu-B1.219 in infected hosts. (e.g., See Logan & Shenk, 1984, 30 Proc. Natl. Acad. Sci. USA 81:3655-3659). Alternatively, the vaccinia 7.5K promoter may be used. (See, e.g., Mackett et al., 1982, Proc. Natl. Acad. Sci. USA 79:7415-7419; Mackett et al., 1984, J. Virol. 49:857-864; Panicali et al., 1982, Proc. Natl. Acad. Sci. USA 79:4927-4931).

35 Specific initiation signals may also be required for efficient translation of inserted Hu-B1.219 coding sequences. These signals include the ATG initiation codon and adjacent

sequences. In cases where the entire Hu-B1.219 gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed.

- 5 However, in cases where only a portion of the Hu-B1.219 coding sequence is inserted, exogenous translational control signals, including the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the Hu-B1.219 coding sequence to  
10 ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription  
15 terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:516-544).

- In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific  
20 fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. The presence of several consensus N-glycosylation sites in the Hu-B1.219 extracellular domain support the possibility that proper  
25 modification may be important for Hu-B1.219 function. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and  
30 processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited  
35 to CHO, VERO, BHK, HeLa, COS, MDCK, 293, WI38, etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell



lines which stably express the Hu-B1.219 may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the Hu-B1.219 DNA controlled by appropriate expression  
5 control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective  
10 media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to  
15 engineer cell lines which express the Hu-B1.219 on the cell surface. Such engineered cell lines are particularly useful in screening for ligands or drugs that affect Hu-B1.219 function.

A number of selection systems may be used, including but  
20 not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817)  
25 genes can be employed in tk<sup>-</sup>, hgp<sup>r</sup>t<sup>-</sup> or ap<sup>r</sup>t<sup>-</sup> cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for *dhfr*, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA  
30 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981), Proc. Natl. Acad. Sci. USA 78:2072); *neo*, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and *hygro*, which confers resistance to hygromycin (Santerre, et  
35 al., 1984, Gene 30:147) genes. Recently, additional selectable genes have been described, namely *trpB*, which allows cells to utilize indole in place of tryptophan; *hisD*,

which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, 1988, Proc. Natl. Acad. Sci. USA 85:8047); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue L., 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.).

#### 5.4. IDENTIFICATION OF CELLS THAT EXPRESS Hu-B1.219

10 The host cells which contain the coding sequence and which express the biologically active gene product may be identified by at least four general approaches; (a) DNA-DNA or DNA-RNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of  
15 transcription as measured by the expression of Hu-B1.219 mRNA transcripts in the host cell; and (d) detection of the gene product as measured by immunoassay or by its biological activity. Prior to the identification of gene expression, the host cells may be first mutagenized in an effort to  
20 increase the level of expression of Hu-B1.219, especially in cell lines that produce low amounts of Hu-B1.219.

In the first approach, the presence of the Hu-B1.219 coding sequence inserted in the expression vector can be detected by DNA-DNA or DNA-RNA hybridization using probes  
25 comprising nucleotide sequences that are homologous to the Hu-B1.219 coding sequence, respectively, or portions or derivatives thereof.

In the second approach, the recombinant expression vector/host system can be identified and selected based upon  
30 the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the Hu-B1.219 coding sequence is inserted within a marker  
35 gene sequence of the vector, recombinants containing the Hu-B1.219 coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can

be placed in tandem with the Hu-B1.219 sequence under the control of the same or different promoter used to control the expression of the Hu-B1.219 coding sequence. Expression of the marker in response to induction or selection indicates  
5 expression of the Hu-B1.219 coding sequence.

In the third approach, transcriptional activity for the Hu-B1.219 coding region can be assessed by hybridization assays. For example, RNA can be isolated and analyzed by Northern blot using a probe homologous to the Hu-B1.219  
10 coding sequence or particular portions thereof. Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of the Hu-B1.219 protein product can be assessed immunologically, for example  
15 by Western blots, immunoassays such as radioimmuno-precipitation, enzyme-linked immunoassays and the like.

#### 5.5. USES OF Hu-B1.219 ENGINEERED CELL LINES

In an embodiment of the invention, the Hu-B1.219 receptor and/or cell lines that express the Hu-B1.219  
20 receptor may be used to screen for antibodies, peptides, or other ligands that act as agonists or antagonists of the Hu-B1.219 receptor. For example, anti-Hu-B1.219 antibodies may be used to inhibit or stimulate receptor Hu-B1.219 function. Alternatively, screening of peptide libraries with  
25 recombinantly expressed soluble Hu-B1.219 protein or cell lines expressing Hu-B1.219 protein may be useful for identification of therapeutic molecules that function by inhibiting or stimulating the biological activity of Hu-B1.219. The uses of the Hu-B1.219 receptor and engineered  
30 cell lines, described in the subsections below, may be employed equally well for other members of the HR family.

In an embodiment of the invention, engineered cell lines which express most of the Hu-B1.219 coding region or its ligand binding domain or its ligand binding domain fused to  
35 another molecule such as the immunoglobulin constant region (Hollenbaugh and Aruffo, 1992, Current Protocols in Immunology, Unit 10.19; Aruffo et al., 1990, Cell 61:1303)

may be utilized to produce a soluble receptor to screen and identify ligand antagonists as well as agonists. The soluble Hu-B1.219 protein or fusion protein may be used to identify a ligand in binding assays, affinity chromatography, immunoprecipitation, Western blot, and the like. Alternatively, the ligand binding domain of Hu-B1.219 may be fused to the coding sequence of the epidermal growth factor receptor transmembrane and cytoplasmic regions. This approach provides for the use of the epidermal growth factor receptor signal transduction pathway as a means for detecting ligands that bind to Hu-B1.219 in a manner capable of triggering an intracellular signal. Synthetic compounds, natural products, and other sources of potentially biologically active materials can be screened in a number of ways.

Random peptide libraries consisting of all possible combinations of amino acids attached to a solid phase support may be used to identify peptides that are able to bind to the ligand binding site of a given receptor or other functional domains of a receptor such as kinase domains (Lam, K.S. et al., 1991, Nature 354: 82-84). The screening of peptide libraries may have therapeutic value in the discovery of pharmaceutical agents that stimulate or inhibit the biological activity of receptors through their interactions with the given receptor.

Identification of molecules that are able to bind to the Hu-B1.219 may be accomplished by screening a peptide library with recombinant soluble Hu-B1.219 protein. Methods for expression and purification of Hu-B1.219 are described in Section 5.2, supra, and may be used to express recombinant full length Hu-B1.219 or fragments of Hu-B1.219 depending on the functional domains of interest. For example, the cytoplasmic and extracellular ligand binding domains of Hu-B1.219 may be separately expressed and used to screen peptide libraries.

To identify and isolate the peptide/solid phase support that interacts and forms a complex with Hu-B1.219, it is

necessary to label or "tag" the Hu-B1.219 molecule. The Hu-B1.219 protein may be conjugated to enzymes such as alkaline phosphatase or horseradish peroxidase or to other reagents such as fluorescent labels which may include fluorescein isothiocyanate (FITC), phycoerythrin (PE) or rhodamine. Conjugation of any given label to Hu-B1.219 may be performed using techniques that are routine in the art. Alternatively, Hu-B1.219 expression vectors may be engineered to express a chimeric Hu-B1.219 protein containing an epitope for which a commercially available antibody exist. The epitope specific antibody may be tagged using methods well known in the art including labeling with enzymes, fluorescent dyes or colored or magnetic beads.

The "tagged" Hu-B1.219 conjugate is incubated with the random peptide library for 30 minutes to one hour at 22°C to allow complex formation between Hu-B1.219 and peptide species within the library. The library is then washed to remove any unbound Hu-B1.219 protein. If Hu-B1.219 has been conjugated to alkaline phosphatase or horseradish peroxidase the whole library is poured into a petri dish containing substrates for either alkaline phosphatase or peroxidase, for example, 5-bromo-4-chloro-3-indoyl phosphate (BCIP) or 3,3',4,4'-diaminobenzidine (DAB), respectively. After incubating for several minutes, the peptide/solid phase-Hu-B1.219 complex changes color, and can be easily identified and isolated physically under a dissecting microscope with a micromanipulator. If a fluorescent tagged Hu-B1.219 molecule has been used, complexes may be isolated by fluorescent activated sorting. If a chimeric Hu-B1.219 protein expressing a heterologous epitope has been used, detection of the peptide/Hu-B1.219 complex may be accomplished by using a labeled epitope specific antibody. Once isolated, the identity of the peptide attached to the solid phase support may be determined by peptide sequencing.

In addition to using soluble Hu-B1.219 molecules, in another embodiment, it is possible to detect peptides that bind to cell surface receptors using intact cells. The use

of intact cells is preferred for use with receptors that are multi-subunits or labile or with receptors that require the lipid domain of the cell membrane to be functional. Methods for generating cell lines expressing Hu-B1.219 are described in Section 5.3. The cells used in this technique may be either live or fixed cells. The cells may be incubated with the random peptide library and bind to certain peptides in the library to form a "rosette" between the target cells and the relevant solid phase support/peptide. The rosette can thereafter be isolated by differential centrifugation or removed physically under a dissecting microscope.

As an alternative to whole cell assays for membrane bound receptors or receptors that require the lipid domain of the cell membrane to be functional, the receptor molecules can be reconstituted into liposomes where label or "tag" can be attached.

Various procedures known in the art may be used for the production of antibodies to epitopes of the recombinantly produced Hu-B1.219 receptor. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression library. Neutralizing antibodies i.e., those which compete for the ligand binding site of the receptor are especially preferred for diagnostics and therapeutics.

Monoclonal antibodies that bind Hu-B1.219 may be radioactively labeled allowing one to follow their location and distribution in the body after injection. Radioisotope tagged antibodies may be used as a non-invasive diagnostic tool for imaging *de novo* cells of tumors and metastases.

Immunotoxins may also be designed which target cytotoxic agents to specific sites in the body. For example, high affinity Hu-B1.219 specific monoclonal antibodies may be covalently complexed to bacterial or plant toxins, such as diphtheria toxin, abrin or ricin. A general method of preparation of antibody/hybrid molecules may involve use of thiol-crosslinking reagents such as SPDP, which attack the primary amino groups on the antibody and by disulfide

exchange, attach the toxin to the antibody. The hybrid antibodies may be used to specifically eliminate Hu-B1.219 expressing tumor cells.

For the production of antibodies, various host animals may be immunized by injection with the Hu-B1.219 protein including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

Monoclonal antibodies to Hu-B1.219 may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, (Nature, 1975, 256:495-497), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today, 4:72; Cote et al., 1983, Proc. Natl. Acad. Sci., 80:2026-2030) and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce Hu-B1.219-specific single chain antibodies.

Antibody fragments which contain specific binding sites of Hu-B1.219 may be generated by known techniques. For

example, such fragments include but are not limited to: the F(ab')<sub>2</sub> fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity to Hu-B1.219.

#### 5.6. USES OF Hu-B1.219 POLYNUCLEOTIDE

10 An Hu-B1.219 polynucleotide may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, an Hu-B1.219 polynucleotide may be used to detect Hu-B1.219 gene expression or aberrant Hu-B1.219 gene expression in disease states, e.g., chronic myelogenous leukemia. Included in the  
15 scope of the invention are oligonucleotide sequences, that include antisense RNA and DNA molecules and ribozymes, that function to inhibit translation of an Hu-B1.219.

##### 5.6.1. DIAGNOSTIC USES OF AN Hu-B1.219 POLYNUCLEOTIDE

20 An Hu-B1.219 polynucleotide may have a number of uses for the diagnosis of diseases resulting from aberrant expression of Hu-B1.219. For example, the Hu-B1.219 DNA sequence may be used in hybridization assays of biopsies or autopsies to diagnose abnormalities of Hu-B1.219 expression;  
25 e.g., Southern or Northern analysis, including *in situ* hybridization assays. Such techniques are well known in the art, and are in fact the basis of many commercially available diagnostic kits.

##### 5.6.2. THERAPEUTIC USES OF AN Hu-B1.219 POLYNUCLEOTIDE

30 An Hu-B1.219 polynucleotide may be useful in the treatment of various abnormal conditions. By introducing gene sequences into cells, gene therapy can be used to treat conditions in which the cells do not proliferate or differentiate normally due to underexpression of normal Hu-B1.219 or expression of abnormal/inactive Hu-B1.219. In some  
35 instances, the polynucleotide encoding an Hu-B1.219 is



intended to replace or act in the place of a functionally deficient endogenous gene. Alternatively, abnormal conditions characterized by overproliferation can be treated using the gene therapy techniques described below.

5 Abnormal cellular proliferation is an important component of a variety of disease states. Recombinant gene therapy vectors, such as viral vectors, may be engineered to express variant, signalling incompetent forms of Hu-B1.219 which may be used to inhibit the activity of the naturally  
10 occurring endogenous Hu-B1.219. A signalling incompetent form may be, for example, a truncated form of the protein that is lacking all or part of its signal transduction domain. Such a truncated form may participate in normal binding to a substrate but lack signal transduction activity.  
15 Thus recombinant gene therapy vectors may be used therapeutically for treatment of diseases resulting from aberrant expression or activity of an Hu-B1.219. Accordingly, the invention provides a method of inhibiting the effects of signal transduction by an endogenous Hu-B1.219  
20 protein in a cell comprising delivering a DNA molecule encoding a signalling incompetent form of the Hu-B1.219 protein to the cell so that the signalling incompetent Hu-B1.219 protein is produced in the cell and competes with the endogenous Hu-B1.219 protein for access to molecules in the  
25 Hu-B1.219 protein signalling pathway which activate or are activated by the endogenous Hu-B1.219 protein.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery  
30 of recombinant Hu-B1.219 into the targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors containing an Hu-B1.219 polynucleotide sequence. See, for example, the techniques described in Maniatis et al., 1989, Molecular  
35 Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience,

N.Y. Alternatively, recombinant Hu-B1.219 molecules can be reconstituted into liposomes for delivery to target cells.

Oligonucleotide sequences, that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of an Hu-B1.219 mRNA are within the scope of the invention. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between -10 and +10 regions of an Hu-B1.219 nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of Hu-B1.219 RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA

molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase 5 promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be 10 introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than 15 phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

Methods for introducing polynucleotides into such cells or tissue include methods for *in vitro* introduction of polynucleotides such as the insertion of naked 20 polynucleotide, *i.e.*, by injection into tissue, the introduction of an Hu-B1.219 polynucleotide in a cell *ex vivo*, *i.e.*, for use in autologous cell therapy, the use of a vector such as a virus, retrovirus, phage or plasmid, etc. or techniques such as electroporation which may be used *in vivo* 25 or *ex vivo*.

## 6. **EXAMPLE: MOLECULAR CLONING OF A NOVEL HEMATOPOIETIN RECEPTOR COMPLEMENTARY DNA**

### 6.1. **MATERIALS AND METHODS**

#### 30 6.1.1. **NORTHERN BLOT ANALYSIS**

In order to study the expression of the Hu-B1.219 gene, Northern blots containing RNA obtained from a variety of human tissues (Clontech, Palo Alto, CA) were hybridized with a radiolabeled 530 base pair (bp) DNA probe corresponding to 35 nucleotides #578 through 1107 (see Figure 2A-2G). Briefly, the blots were prehybridized at 42°C for 3-6 hours in a solution containing 5X SSPE, 10X Denhardt's solution, 100

µg/ml freshly denatured, sheared salmon sperm DNA, 50% formamide (freshly deionized), and 2% SDS. The radiolabeled probe was heat denatured and added to the prehybridization mix and allowed to hybridize at 42°C for 18-24 hours with 5 constant shaking. The blots were rinsed in 2X SSC, 0.05% SDS several times at room temperature before being transferred to a wash solution containing 0.1X SSC, 0.1% SDS and agitated at 50°C for 40 minutes. The blots were then covered with plastic wrap, mounted on Whatman paper and exposed to x-ray 10 film at -70°C using an intensifying screen.

#### 6.1.2. REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION (RT/PCR)

Total RNA was isolated using standard laboratory procedures (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, NY). 15 Approximately 1 µg of total RNA was reverse transcribed and the cDNA was amplified by PCR (Perkin Elmer, Norwalk, CT). The PCR amplification conditions were the same for Hu-B1.219 and Form 1 expression analysis. They were: 94°C for 30 sec, 20 60°C for 30 sec, 72°C for 30 sec for a total of 40 cycles. The amplified products (224 bp for Hu-B1.219 and 816 bp for Form 1) were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. The Hu-B1.219 amplimers were GTTTGCATATGGAAGTC (upper) and 25 CCTGAACCATCCAGTCTCT (lower). The Form 1 specific amplimers were GACTCATTGTGCAGTGTTTCAG (upper) and TAGTGAGGGGAGGGTCAGCAG (lower). The upper amplimer was commonly shared by all 3 forms, whereas the lower amplimer was Form 1-specific.

#### 6.2. RESULTS

30 A number of cDNA clones were isolated from a human fetal liver cDNA library (Clontech, Palo Alto, CA), and the DNA sequences of several of these clones were determined. These clones (Hu-B1.219 #4, #33, #34, #1, #36, #8, #55, #60, #3, 35 #57, #62) contained overlapping sequences, which were then compiled into a contiguous nucleotide sequence. Both the cDNA sequence and predicted protein sequence from the cDNA are shown in Figure 2A-2G. This cDNA sequence contains two

FN III domains, each containing a "WS box", which are characteristic of genes of the HR family. However, the Hu-B1.219 sequence is not identical to any known gene. Thus, this cDNA represents a novel member of the HR gene family, 5 herein referred to as Hu-B1.219 (Table 1).

Table 1  
Cytokine Receptor Gene FN III Domain Sizes (bp)

Gene	Human	Mouse	Rat
Hu-B1.219 (5')	273		
Hu-B1.219 (3')	282		
IL-2R $\beta$	291	288	291
IL-2R $\gamma$	273		
IL-3R $\alpha$	246	252	
IL-3R $\beta$ Aic2a		306 and 273	
IL-3R $\beta$ Aic2b	306 and 282	303 and 276	
IL-4R	294		291
IL-5R $\alpha$	276	273	
IL-6R	288	285	
gp130	288	291	288
IL-7R		294	
IL-9R	321	321	
mpl		270	
G-CSFR	300	297	
GM-CSFR	288		
CNTFR	282		285
PRLR			288
EPOR	288	285	288
LIFR-1	321 and 297		

Based on the sequence of Hu-B1.219 presented in Figure 2A-2G, the translation initiation site appears at position #97. The sequence encodes an open reading frame up to and

including nucleotide #2970. It is believed that the sequence between nucleotides #2614 and #2691 encodes a transmembrane domain. The complete sequence encodes a protein of 958 amino acids.

5        However, the sequence in Figure 2A-2G represents only one form of Hu-B1.219 cDNA sequence, herein referred to as Form 1. This is because additional lambda clones were discovered that contained different sequences near the 3' end known as Form 2 and Form 3. All three forms contain the  
10 identical sequence up to and including nucleotide #2770, then they diverge at nucleotide #2771 and beyond (Figure 3A). An alignment of deduced amino acid sequences of all three forms corresponding to the 3' end from #2771 until a stop codon is shown in Figure 3B. Two of the originally isolated lambda  
15 clones, #36 and #8, contain the 3' end sequences of Form 1 and Form 2, respectively. These three forms of Hu-B1.219 may derive from a common precursor mRNA by an alternative splicing mechanism.

It is noteworthy that the DNA sequence of Form 1 from  
20 nucleotide #2771 to the end is 98% identical to a human retrotransposon sequence that is thought to be derived from a human endogenous retroviral DNA sequence (Singer, 1982, Cell 28:433; Weiner et al., 1986, Ann. Rev. Biochem. 55:631; Lower et al., 1993, Proc. Natl. Acad. Sci. USA 90:4480). In order  
25 to examine the expression of the different forms of cDNA, RT/PCR was performed using several human cell lines. The results in Table 2 show that Form 1 was expressed as RNA in K-562 cells and in a human fetal liver cDNA preparation. Since Hu-B1.219 was cloned from human fetal liver cDNA  
30 library, this served as a positive control. However, with respect to several other human cell lines, Form 1 was not detected, whereas Hu-B1.219 expression was positive. For example, Form 1 was not expressed in KG1a cells, but Form 3 was expressed. Thus, it is possible that these three forms  
35 of Hu-B1.219 are not expressed simultaneously in the same cells. There may be selective expression of certain forms in particular cell populations.

Table 2  
RT/PCR Analysis of Hu-B1.219 Expression

	Cell Lines	Hu-B1.219*	Form 1 $\Delta$	Form 3 $\Delta$
5	MRC5 (Lung fibroblast)	++	+/-	+
	KG1a (lymphoblast)	+	-	++
	Raji (B cell lymphoma)	+	-	+
	Kit 225/K6 (T cell)	+++	-	+
	K562 (myelogenous leukemia)	++++	+++	++++
10	Human Fetal Liver (positive control)	+++	+++	+++

\* - Analysis by Northern blots

$\Delta$  - Analysis by RT/PCR

15 Various human tissue RNA were probed with a  
radiolabelled Hu-B1.219 fragment corresponding to nucleotide  
numbers from #578 to #1107 as disclosed in Figure 2A-2G for  
Northern blot analyses. Two different size mRNAs were  
detected. This result suggests that there may be another  
homologous gene or there is alternative splicing of a single  
20 RNA transcript. Hu-B1.219 expression was by far the  
strongest in human fetal tissues, particularly the liver and  
lung. Trace levels were found in several adult tissues.  
Interestingly, a chronic myelogenous leukemia cell line,  
K562, was strongly positive for its expression, while some  
25 expression was also detected in A549 cells, a lung carcinoma  
cell line (Table 3).

30

35

Table 3  
SUMMARY OF NORTHERN BLOT ANALYSIS OF  
Hu-B1.219 GENE EXPRESSION

	<u>Human Tissues/cell lines</u>	<u>Expression</u>
5	fetal brain	-
	lung	+++
	liver	+++++
	kidney	+
	adult heart	+
10	brain	-
	placenta	+/-
	lung	+
	liver	+
	skeletal muscle	-
	kidney	+/-
	pancreas	-
15	spleen	-
	thymus	-
	prostate	-
	testis	-
	ovary	+
	small intestine	-
	colon	-
	peripheral blood	-
20	leukocytes	-
	cancer HL-60	-
	HeLa	-
	K-562	+++
	MOLT-4	-
	Raji	-
	SW480	-
25	A549	+
	G361	-

Taken together, the data indicates that the Hu-B1.219 cDNA clone represents a new member of the human hematopoietin receptor family. A summary of the data that supports this conclusion is as follows:

1. The Hu-B1.219 DNA and protein sequences do not fully match any known sequences in the corresponding computer data bases.
2. Hu-B1.219 shares certain DNA sequence homology with the IL-6R and IL-4R.
3. It shares certain protein homology with G-CSFR, IL-6R, IL-3R beta chain, gp130, IL-12R, and LIFR.



4. It contains two "WS box" motifs with the correct spacing of conserved amino acids in the FN III domains (see Figure 4).

5. It contains an amphipathic sequence in block 3 of the FN III domains (see Figure 5).

6. It contains alternating hydrophobic and basic amino acids in block 6 of the FN III domains (see Figure 6).

7. It contains conserved cysteines in these cysteine rich regions upstream of the FN III domains.

10 8. It was originally cloned from a hematopoietic tissue, fetal liver.

9. It is expressed by certain fetal tissues.

#### 7. Deposit of Microorganisms

15 The following organisms were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

#### Strain Designation      Accession No.

	HuB1.219, #1	75885
20	HuB1.219, #4	75886
	HuB1.219, #8	75887
	HuB1.219, #33	75888
	HuB1.219, #34	75889
	HuB1.219, #36	75890
	HuB1.219, #55	75971
	HuB1.219, #60	75973
	HuB1.219, #3	75970
25	HuB1.219, #57	75972
	HuB1.219, #62	75974

The present invention is not to be limited in scope by the exemplified embodiments, which are intended as  
 30 illustrations of individual aspects of the invention.  
 Indeed, various modifications for the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to  
 35 fall within the scope of the appended claims.

All publications cited herein are incorporated by reference in their entirety.

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## SEQUENCE LISTIN

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(ii) TITLE OF INVENTION: Hu-B1.219, A NOVEL HUMAN HEMATOPOIETIN RECEPTOR

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(E) COUNTRY: USA  
(F) ZIP: 10036-2711

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/355,888  
(B) FILING DATE: 14-DEC-1994  
(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Poissant, Brian M.  
(B) REGISTRATION NUMBER: 28,462  
(C) REFERENCE/DOCKET NUMBER: 7225-078

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (212) 790-9090  
(B) TELEFAX: (212) 869-9741/8864  
(C) TELEX: 66141 PENNIE

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Trp Ser Xaa Trp Ser  
1 5

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGTTTGCATA TGGAAGTC

18

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTGAACCAT CCACTCTCT

19

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GACTCATTGT GCAGTGTTCA G

21

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TAGTGGAGGG AGGGTCAGCA G

21

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2991 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION: 1..2991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCG CGC GCG ACG CAG GTG CCC GAG CCC CGG CCC GCG CCC ATC TCT GCC	48
Ala Arg Ala Thr Gln Val Pro Glu Pro Arg Pro Ala Pro Ile Ser Ala	
1 5 10 15	
TTC GGT CGA GTT GGA CCC CCG GAT CAA GGT GTA CTT CTC TGA AGT AAG	96
Phe Gly Arg Val Gly Pro Pro Asp Gln Gly Val Leu Leu * Ser Lys	
20 25 30	
ATG ATT TGT CAA AAA TTC TGT GTG GTT TTG TTA CAT TGG GAA TTT ATT	144
Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe Ile	
35 40 45	
TAT GTG ATA ACT GCG TTT AAC TTG TCA TAT CCA ATT ACT CCT TGG AGA	192
Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro Trp Arg	
50 55 60	
TTT AAG TTG TCT TGC ATG CCA CCA AAT TCA ACC TAT GAC TAC TTC CTT	240
Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp Tyr Phe Leu	
65 70 75 80	
TTG CCT GCT GGA CTC TCA AAG AAT ACT TCA AAT TCG AAT GGA CAT TAT	288
Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser Asn Gly His Tyr	
85 90 95	
GAG ACA GCT GTT GAA CCT AAG TTT AAT TCA AGT GGT ACT CAC TTT TCT	336
Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser Gly Thr His Phe Ser	
100 105 110	
AAC TTA TCC AAA GCA ACT TTC CAC TGT TGC TTT CGG AGT GAG CAA GAT	384
Asn Leu Ser Lys Ala Thr Phe His Cys Cys Phe Arg Ser Glu Gln Asp	
115 120 125	
AGA AAC TGC TCC TTA TGT GCA GAC AAC ATT GAA GGA AGG ACA TTT GTT	432
Arg Asn Cys Ser Leu Cys Ala Asp Asn Ile Glu Gly Arg Thr Phe Val	
130 135 140	
TCA ACA GTA AAT TCT TTA GTT TTT CAA CAA ATA GAT GCA AAC TGG AAC	480
Ser Thr Val Asn Ser Leu Val Phe Gln Gln Ile Asp Ala Asn Trp Asn	
145 150 155 160	
ATA CAG TGC TGG CTA AAA GGA GAC TTA AAA TTA TTC ATC TGT TAT GTG	528
Ile Gln Cys Trp Leu Lys Gly Asp Leu Lys Leu Phe Ile Cys Tyr Val	
165 170 175	
GAG TCA TTA TTT AAG AAT CTA TTC AGG AAT TAT AAC TAT AAG GTC CAT	576
Glu Ser Leu Phe Lys Asn Leu Phe Arg Asn Tyr Asn Tyr Lys Val His	
180 185 190	
CTT TTA TAT GTT CTG CCT GAA GTG TTA GAA GAT TCA CCT CTG GTT CCC	624
Leu Leu Tyr Val Leu Pro Glu Val Leu Glu Asp Ser Pro Leu Val Pro	
195 200 205	

CAA Gln 210	AAA Lys	GGC Gly	AGT Ser	TTT Phe	CAG Gln 215	ATG Met	GTT Val	CAC His	TGC Cys	AAT Asn 220	TGC Cys	AGT Ser	GTT Val	CAT His	GAA Glu	672
TGT Cys 225	TGT Cys	GAA Glu	TGT Cys	CTT Leu 230	GTG Val	CCT Pro	GTG Val	CCA Pro	ACA Thr	GCC Ala 235	AAA Lys	CTC Leu	AAC Asn	GAC Asp	ACT Thr 240	720
CTC Leu	CTT Leu	ATG Met	TGT Cys	TTG Leu 245	AAA Lys	ATC Ile	ACA Thr	TCT Ser	GGT Gly 250	GGA Gly	GTA Val	ATT Ile	TTC Phe	CGG Arg 255	TCA Ser	768
CCT Pro	CTA Leu	ATG Met	TCA Ser 260	GTT Val	CAG Gln	CCC Pro	ATA Ile	AAT Asn 265	ATG Met	GTG Val	AAG Lys	CCT Pro	GAT Asp 270	CCA Pro	CCA Pro	816
TTA Leu	GGT Gly 275	TTG Leu	CAT His	ATG Met	GAA Glu	ATC Ile	ACA Thr 280	GAT Asp	GAT Asp	GGT Gly	AAT Asn 285	TTA Leu	AAG Lys	ATT Ile	TCT Ser	864
TGG Trp 290	TCC Ser	AGC Ser	CCA Pro	CCA Pro	TTG Leu 295	GTA Val	CCA Pro	TTT Phe	CCA Pro	CTT Leu	CAA Gln 300	TAT Tyr	CAA Gln	GTG Val	AAA Lys	912
TAT Tyr 305	TCA Ser	GAG Glu	AAT Asn	TCT Ser	ACA Thr 310	ACA Thr	GTT Val	ATC Ile	AGA Arg	GAA Glu 315	GCT Ala	GAC Asp	AAG Lys	ATT Ile	GTC Val 320	960
TCA Ser	GCT Ala	ACA Thr	TCC Ser	CTG Leu 325	CTA Leu	GTA Val	GAC Asp	AGT Ser	ATA Ile 330	CTT Leu	CCT Pro	GGG Gly	TCT Ser	TCG Ser 335	TAT Tyr	1008
GAG Glu	GTT Val	CAG Gln	GTG Val 340	AGG Arg	GGC Gly	AAG Lys	AGA Arg	CTG Leu 345	GAT Asp	GGC Gly	CCA Pro	GGA Gly	ATC Ile 350	TGG Trp	AGT Ser	1056
GAC Asp	TGG Trp	AGT Ser 355	ACT Thr	CCT Pro	CGT Arg	GTC Val	TTT Phe 360	ACC Thr	ACA Thr	CAA Gln	GAT Asp 365	GTC Val	ATA Ile	TAC Tyr	TTT Phe	1104
CCA Pro 370	CCT Pro	AAA Lys	ATT Ile	CTG Leu	ACA Thr	AGT Ser 375	GTT Val	GGG Gly	TCT Ser	AAT Asn 380	GTT Val	TCT Ser	TTT Phe	CAC His	TGC Cys	1152
ATC Ile 385	TAT Tyr	AAG Lys	AAG Lys	GAA Glu 390	AAC Asn	AAG Lys	ATT Ile	GTT Val	CCC Pro	TCA Ser 395	AAA Lys	GAG Glu	ATT Ile	GTT Val	TGG Trp 400	1200
TGG Trp	ATG Met	AAT Asn	TTA Leu	GCT Ala 405	GAG Glu	AAA Lys	ATT Ile	CCT Pro	CAA Gln 410	AGC Ser	CAG Gln	TAT Tyr	GAT Asp	GTT Val 415	GTG Val	1248
AGT Ser	GAT Asp	CAT His	GTT Val 420	AGC Ser	AAA Lys	GTT Val	ACT Thr	TTT Phe 425	TTC Phe	AAT Asn	CTG Leu	AAT Asn	GAA Glu 430	ACC Thr	AAA Lys	1296
CCT Pro	CGA Arg	GGA Gly 435	AAG Lys	TTT Phe	ACC Thr	TAT Tyr	GAT Asp 440	GCA Ala	GTG Val	TAC Tyr	TGC Cys	TGC Cys	AAT Asn	GAA Glu	CAT His	1344
GAA Glu 450	TGC Cys	CAT His	CAT His	CGC Arg	TAT Tyr	GCT Ala 455	GAA Glu	TTA Leu	TAT Tyr	GTG Val	ATT Ile 460	GAT Asp	GTC Val	AAT Asn	ATC Ile	1392
AAT Asn 465	ATC Ile	TCA Ser	TGT Cys	GAA Glu 470	ACT Thr	GAT Asp	GGG Gly	TAC Tyr	TTA Leu 475	ACT Thr	AAA Lys	ATG Met	ACT Thr	TGC Cys	AGA Arg 480	1440

TGG	TCA	ACC	AGT	ACA	ATC	CAG	TCA	CTT	GCG	GAA	AGC	ACT	TTG	CAA	TTG	1488
Trp	Ser	Thr	Ser	Thr	Ile	Gln	Ser	Leu	Ala	Glu	Ser	Thr	Leu	Gln	Leu	
				485					490					495		
AGG	TAT	CAT	AGG	AGC	AGC	CTT	TAC	TGT	TCT	AT	ATT	CCA	TCT	ATT	CAT	1536
Arg	Tyr	His	Arg	Ser	Ser	Leu	Tyr	Cys	Ser	Asp	Ile	Pro	Ser	Ile	His	
			500					505					510			
CCC	ATA	TCT	GAG	CCC	AAA	GAT	TGC	TAT	TTG	CAG	AGT	GAT	GGT	TTT	TAT	1584
Pro	Ile	Ser	Glu	Pro	Lys	Asp	Cys	Tyr	Leu	Gln	Ser	Asp	Gly	Phe	Tyr	
		515					520					525				
GAA	TGC	ATT	TTC	CAG	CCA	ATC	TTC	CTA	TTA	TCT	GGC	TAC	ACA	ATG	TGG	1632
Glu	Cys	Ile	Phe	Gln	Pro	Ile	Phe	Leu	Leu	Ser	Gly	Tyr	Thr	Met	Trp	
	530					535					540					
ATT	AGG	ATC	AAT	CAC	TCT	CTA	GGT	TCA	CTT	GAC	TCT	CCA	CCA	ACA	TGT	1680
Ile	Arg	Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr	Cys	
545					550					555					560	
GTC	CTT	CCT	GAT	TCT	GTG	GTG	AAG	CCA	CTG	CCT	CCA	TCC	AGT	GTG	AAA	1728
Val	Leu	Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Ser	Val	Lys	
				565					570					575		
GCA	GAA	ATT	ACT	ATA	AAC	ATT	GGA	TTA	TTG	AAA	ATA	TCT	TGG	GAA	AAG	1776
Ala	Glu	Ile	Thr	Ile	Asn	Ile	Gly	Leu	Leu	Lys	Ile	Ser	Trp	Glu	Lys	
			580					585					590			
CCA	GTC	TTT	CCA	CAG	AAT	AAC	CTT	CAA	TTC	CAG	ATT	CGC	TAT	GGT	TTA	1824
Pro	Val	Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	Gln	Ile	Arg	Tyr	Gly	Leu	
		595					600					605				
AGT	GGA	AAA	GAA	GTA	CAA	TGG	AAG	ATG	TAT	GAG	GTT	TAT	GAT	GCA	AAA	1872
Ser	Gly	Lys	Glu	Val	Gln	Trp	Lys	Met	Tyr	Glu	Val	Tyr	Asp	Ala	Lys	
	610					615					620					
TCA	AAA	TCT	GTC	AGT	CTC	CCA	GTT	CCA	GAC	TTG	TGT	GCA	GTC	TAT	GCT	1920
Ser	Lys	Ser	Val	Ser	Leu	Pro	Val	Pro	Asp	Leu	Cys	Ala	Val	Tyr	Ala	
625					630					635					640	
GTT	CAG	GTG	CGC	TGT	AAG	AGG	CTA	GAT	GGA	CTG	GGA	TAT	TGG	AGT	AAT	1968
Val	Gln	Val	Arg	Cys	Lys	Arg	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	
			645						650					655		
TGG	AGC	AAT	CCA	GCC	TAC	ACA	GTT	GTC	ATG	GAT	ATA	AAA	GTT	CCT	ATG	2016
Trp	Ser	Asn	Pro	Ala	Tyr	Thr	Val	Val	Met	Asp	Ile	Lys	Val	Pro	Met	
			660					665					670			
AGA	GGA	CCT	GAA	TTT	TGG	AGA	ATA	ATT	AAT	GGA	GAT	ACT	ATG	AAA	AAG	2064
Arg	Gly	Pro	Glu	Phe	Trp	Arg	Ile	Ile	Asn	Gly	Asp	Thr	Met	Lys	Lys	
		675					680					685				
GAG	AAA	AAT	GTC	ACT	TTA	CTT	TGG	AAG	CCC	CTG	ATG	AAA	AAT	GAC	TCA	2112
Glu	Lys	Asn	Val	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Met	Lys	Asn	Asp	Ser	
	690					695					700					
TTG	TGC	AGT	GTT	CAG	AGA	TAT	GTG	ATA	AAC	CAT	CAT	ACT	TCC	TGC	AAT	2160
Leu	Cys	Ser	Val	Gln	Arg	Tyr	Val	Ile	Asn	His	His	Thr	Ser	Cys	Asn	
705					710					715					720	
GGA	ACA	TGG	TCA	GAA	GAT	GTG	GGA	AAT	CAC	ACG	AAA	TTC	ACT	TTC	CTG	2208
Gly	Thr	Trp	Ser	Glu	Asp	Val	Gly	Asn	His	Thr	Lys	Phe	Thr	Phe	Leu	
				725					730					735		
TGG	ACA	GAG	CAA	GCA	CAT	ACT	GTT	ACG	GTT	CTG	GCC	ATC	AAT	TCA	ATT	2256
Trp	Thr	Glu	Gln	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Ile	Asn	Ser	Ile	
			740					745					750			

GGT Gly	GCT Ala	TCT Ser	GTT Val	GCA Ala	AAT Asn	TTT Phe	AAT Asn	TTA Leu	ACC Thr	TTT Phe	TCA Ser	TGG Trp	CCT Pro	ATG Met	AGC Ser	2304
		755					760					765				
AAA Lys	GTA Val	AAT Asn	ATC Ile	GTG Val	CAG Gln	TCA Ser	CTC Leu	AGT Ser	GCT Ala	TAT Tyr	CCT Pro	TTA Leu	AAC Asn	AGC Ser	AGT Ser	2352
		770				775					780					
TGT Cys	GTG Val	ATT Ile	GTT Val	TCC Ser	TGG Trp	ATA Ile	CTA Leu	TCA Ser	CCC Pro	AGT Ser	GAT Asp	TAC Tyr	AAG Lys	CTA Leu	ATG Met	2400
		785			790					795					800	
TAT Tyr	TTT Phe	ATT Ile	ATT Ile	GAG Glu	TGG Trp	AAA Lys	AAT Asn	CTT Leu	AAT Asn	GAA Glu	GAT Asp	GGT Gly	GAA Glu	ATA Ile	AAA Lys	2448
				805					810					815		
TGG Trp	CTT Leu	AGA Arg	ATC Ile	TCT Ser	TCA Ser	TCT Ser	GTT Val	AAG Lys	AAG Lys	TAT Tyr	TAT Tyr	ATC Ile	CAT His	GAT Asp	CAT His	2496
			820					825					830			
TTT Phe	ATC Ile	CCC Pro	ATT Ile	GAG Glu	AAG Lys	TAC Tyr	CAG Gln	TTC Phe	AGT Ser	CTT Leu	TAC Tyr	CCA Pro	ATA Ile	TTT Phe	ATG Met	2544
		835					840					845				
GAA lu	GGA Gly	GTG Val	GGA Gly	AAA Lys	CCA Pro	AAG Lys	ATA Ile	ATT Ile	AAT Asn	AGT Ser	TTC Phe	ACT Thr	CAA Gln	GAT Asp	GAT Asp	2592
		850				855					860					
ATT Ile	GAA Glu	AAA Lys	CAC His	CAG Gln	AGT Ser	GAT Asp	GCA Ala	GGT Gly	TTA Leu	TAT Tyr	GTA Val	ATT Ile	GTG Val	CCA Pro	GTA Val	2640
					870					875					880	
ATT Ile	ATT Ile	TCC Ser	TCT Ser	TCC Ser	ATC Ile	TTA Leu	TTG Leu	CTT Leu	GGA Gly	ACA Thr	TTA Leu	TTA Leu	ATA Ile	TCA Ser	CAC His	2688
				885					890					895		
CAA Gln	AGA Arg	ATG Met	AAA Lys	AAG Lys	CTA Leu	TTT Phe	TGG Trp	GAA Glu	GAT Asp	GTT Val	CCG Pro	AAC Asn	CCC Pro	AAG Lys	AAT Asn	2736
			900					905					910			
TGT Cys	TCC Ser	TGG Trp	GCA Ala	CAA Gln	GGA Gly	CTT Leu	AAT Asn	TTT Phe	CAG Gln	AAG Lys	ATG Met	CTT Leu	GAA Glu	GGC Gly	AGC Ser	2784
		915					920					925				
ATG Met	TTC Phe	GTT Val	AAG Lys	AGT Ser	CAT His	CAC His	CAC His	TCC Ser	CTA Leu	ATC Ile	TCA Ser	AGT Ser	ACC Thr	CAG Gln	GGA Gly	2832
		930				935					940					
CAC His	AAA Lys	CAC His	TGC Cys	GGA Gly	AGG Arg	CCA Pro	CAG Gln	GGT Gly	CCT Pro	CTG Leu	CAT His	AGG Arg	AAA Lys	ACC Thr	AGA Arg	2880
					950				955						960	
GAC Asp	CTT Leu	TGT Cys	TCA Ser	CTT Leu	GTT Val	TAT Tyr	CTG Leu	CTG Leu	ACC Thr	CTC Leu	CCT Pro	CCA Pro	CTA Leu	TTG Leu	TCC Ser	2928
				965					970					975		
TAT Tyr	GAC Asp	CCT Pro	GCC Ala	AAA Lys	TCC Ser	CCC Pro	TCT Ser	GTG Val	AGA Arg	AAC Asn	ACC Thr	CAA Gln	GAA Glu	TGA *	TCA Ser	2976
			980					985					990			
ATA Ile	AAA Lys	AAA Lys	AAA Lys	AAA Lys												2991
			995													

(2) INFORMATION FOR SEQ ID NO:7:



## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 997 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Arg Ala Thr Gln Val Pro Glu Pro Arg Pro Ala Pro Ile Ser Ala  
 1 5 10 15  
 Phe Gly Arg Val Gly Pro Pro Asp Gln Gly Val Leu Leu \* Ser Lys  
 20 25 30  
 Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe Ile  
 35 40 45  
 Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro Trp Arg  
 50 55 60  
 Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp Tyr Phe Leu  
 65 70 75 80  
 Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser Asn Gly His Tyr  
 85 90 95  
 Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser Gly Thr His Phe Ser  
 100 105 110  
 Asn Leu Ser Lys Ala Thr Phe His Cys Cys Phe Arg Ser Glu Gln Asp  
 115 120 125  
 Arg Asn Cys Ser Leu Cys Ala Asp Asn Ile Glu Gly Arg Thr Phe Val  
 130 135 140  
 Ser Thr Val Asn Ser Leu Val Phe Gln Gln Ile Asp Ala Asn Trp Asn  
 145 150 155 160  
 Ile Gln Cys Trp Leu Lys Gly Asp Leu Lys Leu Phe Ile Cys Tyr Val  
 165 170 175  
 Glu Ser Leu Phe Lys Asn Leu Phe Arg Asn Tyr Asn Tyr Lys Val His  
 180 185 190  
 Leu Leu Tyr Val Leu Pro Glu Val Leu Glu Asp Ser Pro Leu Val Pro  
 195 200 205  
 Gln Lys Gly Ser Phe Gln Met Val His Cys Asn Cys Ser Val His Glu  
 210 215 220  
 Cys Cys Glu Cys Leu Val Pro Val Pro Thr Ala Lys Leu Asn Asp Thr  
 225 230 235 240  
 Leu Leu Met Cys Leu Lys Ile Thr Ser Gly Gly Val Ile Phe Arg Ser  
 245 250 255  
 Pro Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro  
 260 265 270  
 Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile Ser  
 275 280 285  
 Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln Val Lys  
 290 295 300  
 Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp Lys Ile Val

305		310		315		320
Ser Ala Thr Ser	Leu 325	Leu Val Asp Ser	Ile 330	Leu Pro Gly Ser	Ser 335	Tyr
Glu Val Gln Val	Arg 340	Gly Lys Arg Leu	Asp 345	Gly Pr Gly Ile	Trp 350	Ser
Asp Trp Ser 355	Thr Pro Arg Val	Phe 360	Thr Thr Gln Asp	Val 365	Ile Tyr Phe	
Pro Pro Lys Ile	Leu Thr Ser 375	Val Gly Ser Asn	Val 380	Ser Phe His Cys		
Ile Tyr Lys Lys	Glu 390	Asn Lys Ile Val	Pro Ser 395	Lys Glu Ile Val	Trp 400	
Trp Met Asn Leu	Ala 405	Glu Lys Ile Pro	Gln 410	Ser Gln Tyr Asp	Val 415	Val
Ser Asp His Val	Ser 420	Lys Val Thr Phe	Phe 425	Asn Leu Asn Glu	Thr 430	Lys
Pro Arg Gly Lys	Phe Thr Tyr Asp	Ala 440	Val Tyr Cys Cys	Asn 445	Glu His	
Glu Cys His His	Arg Tyr Ala 455	Glu Leu Tyr Val	Ile 460	Asp Val Asn Ile		
Asn Ile Ser Cys	Glu 470	Thr Asp Gly Tyr	Leu Thr 475	Lys Met Thr Cys	Arg 480	
Trp Ser Thr Ser	Thr 485	Ile Gln Ser Leu	Ala 490	Glu Ser Thr Leu	Gln 495	Leu
Arg Tyr His Arg	Ser 500	Ser Leu Tyr Cys	Ser 505	Asp Ile Pro Ser	Ile 510	His
Pro Ile Ser Glu	Pro 515	Lys Asp Cys Tyr	Leu Gln Ser	Asp 525	Gly Phe Tyr	
Glu Cys Ile Phe	Gln Pro 535	Ile Phe Leu Leu	Ser 540	Gly Tyr Thr Met	Trp	
Ile Arg Ile Asn	His 550	Ser Leu Gly Ser	Leu Asp 555	Ser Pro Pro Thr	Cys 560	
Val Leu Pro Asp	Ser 565	Val Val Lys Pro	Leu Pro 570	Pro Ser Ser Val	Lys 575	
Ala Glu Ile Thr	Ile 580	Asn Ile Gly Leu	Leu 585	Lys Ile Ser Trp	Glu 590	Lys
Pro Val Phe Pro	Glu 595	Asn Asn Leu Gln	Phe 600	Gln Ile Arg Tyr	Gly 605	Leu
Ser Gly Lys Glu	Val 610	Gln Trp Lys Met	Tyr Glu 620	Val Tyr Asp Ala	Lys	
Ser Lys Ser Val	Ser 625	Leu Pro Val Pro	Asp 635	Leu Cys Ala Val	Tyr 640	Ala
Val Gln Val Arg	Cys 645	Lys Arg Leu Asp	Gly 650	Leu Gly Tyr Trp	Ser 655	Asn
Trp Ser Asn Pro	Ala 660	Tyr Thr Val Val	Met 665	Asp Ile Lys Val	Pro 670	Met

Arg Gly Pr Glu Phe Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys  
 675 680 685  
 Glu Lys Asn Val Thr Leu Leu Trp Lys Pro Leu Met Lys Asn Asp Ser  
 690 695 700  
 Leu Cys Ser Val Gln Arg Tyr Val Ile Asn His His Thr Ser Cys Asn  
 705 710 715 720  
 Gly Thr Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr Phe Leu  
 725 730 735  
 Trp Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn Ser Ile  
 740 745 750  
 Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser  
 755 760 765  
 Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn Ser Ser  
 770 775 780  
 Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr Lys Leu Met  
 785 790 795 800  
 Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp Gly Glu Ile Lys  
 805 810 815  
 Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr Tyr Ile His Asp His  
 820 825 830  
 Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Ile Phe Met  
 835 840 845  
 Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Ser Phe Thr Gln Asp Asp  
 850 855 860  
 Ile Glu Lys His Gln Ser Asp Ala Gly Leu Tyr Val Ile Val Pro Val  
 865 870 875 880  
 Ile Ile Ser Ser Ser Ile Leu Leu Leu Gly Thr Leu Leu Ile Ser His  
 885 890 895  
 Gln Arg Met Lys Lys Leu Phe Trp Glu Asp Val Pro Asn Pro Lys Asn  
 900 905 910  
 Cys Ser Trp Ala Gln Gly Leu Asn Phe Gln Lys Met Leu Glu Gly Ser  
 915 920 925  
 Met Phe Val Lys Ser His His His Ser Leu Ile Ser Ser Thr Gln Gly  
 930 935 940  
 His Lys His Cys Gly Arg Pro Gln Gly Pro Leu His Arg Lys Thr Arg  
 945 950 955 960  
 Asp Leu Cys Ser Leu Val Tyr Leu Leu Thr Leu Pro Pro Leu Leu Ser  
 965 970 975  
 Tyr Asp Pro Ala Lys Ser Pro Ser Val Arg Asn Thr Gln Glu \* Ser  
 980 985 990  
 Ile Lys Lys Lys Lys  
 995

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 241 base pairs

- (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 2..241

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

A GGA CTT AAT TTT CAG AAG ATG CTT GAA GGC AGC ATG TTC GTT AAG	46
Gly Leu Asn Phe Gln Lys Met Leu Glu Gly Ser Met Phe Val Lys	
1 5 10 15	
AGT CAT CAC CAC TCC CTA ATC TCA AGT ACC CAG GGA CAC AAA CAC TGC	94
Ser His His His Ser Leu Ile Ser Ser Thr Gln Gly His Lys His Cys	
20 25 30	
GGA AGG CCA CAG GGT CCT CTG CAT AGG AAA ACC AGA GAC CTT TGT TCA	142
Gly Arg Pro Gln Gly Pro Leu His Arg Lys Thr Arg Asp Leu Cys Ser	
35 40 45	
CTT GTT TAT CTG CTG ACC CTC CCT CCA CTA TTG TCC TAT GAC CCT GCC	190
Leu Val Tyr Leu Leu Thr Leu Pro Pro Leu Leu Ser Tyr Asp Pro Ala	
50 55 60	
AAA TCC CCC TCT GTG AGA AAC ACC CAA GAA TGA TCA ATA AAA AAA AAA	238
Lys Ser Pro Ser Val Arg Asn Thr Gln Glu * Ser Ile Lys Lys Lys	
65 70 75	
AAA	241
Lys	
80	

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 80 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Leu Asn Phe Gln Lys Met Leu Glu Gly Ser Met Phe Val Lys Ser
1 5 10 15
His His His Ser Leu Ile Ser Ser Thr Gln Gly His Lys His Cys Gly
20 25 30
Arg Pro Gln Gly Pro Leu His Arg Lys Thr Arg Asp Leu Cys Ser Leu
35 40 45
Val Tyr Leu Leu Thr Leu Pro Pro Leu Leu Ser Tyr Asp Pro Ala Lys
50 55 60
Ser Pro Ser Val Arg Asn Thr Gln Glu * Ser Ile Lys Lys Lys Lys
65 70 75 80

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 130 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 2..130

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

A	GGA	CTT	AAT	TTT	CAG	AAG	AAA	ATG	CCT	GGC	ACA	AAG	GAA	CTA	CTG		46
	Gly	Leu	Asn	Phe	Gln	Lys	Lys	Met	Pro	Gly	Thr	Lys	Glu	Leu	Leu		
	1				5				10					15			
GGT	GGA	GGT	TGG	TTG	ACT	TAG	GAA	ATG	CTT	GTG	AAG	CTA	CGT	CCT	ACC		94
Gly	Gly	Gly	Trp	Leu	Thr	*	Glu	Met	Leu	Val	Lys	Leu	Arg	Pro	Thr		
			20						25					30			
TCG	TGC	GCA	CCT	GCT	CTC	CCT	GAG	GTG	TGC	ACA	ATG						130
Ser	Cys	Ala	Pro	Ala	Leu	Pro	Glu	Val	Cys	Thr	Met						
			35						40								

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 43 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly	Leu	Asn	Phe	Gln	Lys	Lys	Met	Pro	Gly	Thr	Lys	Glu	Leu	Leu	Gly	
1				5					10					15		
Gly	Gly	Trp	Leu	Thr	*	Glu	Met	Leu	Val	Lys	Leu	Arg	Pro	Thr	Ser	
			20						25					30		
Cys	Ala	Pro	Ala	Leu	Pro	Glu	Val	Cys	Thr	Met						
			35						40							

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 127 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 2..127

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

A GGA CTT AAT TTT CAG AAG AGA ACG GAC ATT CTT TGA AGT CTA ATC 46  
 Gly Leu Asn Phe Gln Lys Arg Thr Asp Ile Leu \* Ser Leu Ile 15  
 1 5 10

ATG ATC ACT ACA GAT AA CCC AAT GTG CCA ACT TCC CAA CAG TCT ATA 94  
 Met Ile Thr Thr Asp Glu Pro Asn Val Pro Thr Ser Gln In Ser Ile 30  
 20 25

GAG TAT TAG AAG ATT TTT ACA TTC TGA AGA AGG 127  
 Glu Tyr \* Lys Ile Phe Thr Phe \* Arg Arg 40  
 35

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gly Leu Asn Phe Gln Lys Arg Thr Asp Ile Leu \* Ser Leu Ile Met  
 1 5 10 15  
 Ile Thr Thr Asp Glu Pro Asn Val Pro Thr Ser Gln Gln Ser Ile Glu  
 20 25 30  
 Tyr \* Lys Ile Phe Thr Phe \* Arg Arg  
 35 40

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Pro Tyr Leu Glu Phe Glu Ala Arg Arg Leu Leu  
 1 5 10

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu His Leu Val Gln Tyr Arg Thr Asp Trp Asp His Ser

1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp His Cys Phe Asn Tyr Glu Leu Lys Ile Tyr Asn Thr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Thr Thr His Ile Arg Tyr Glu Val Asp Val Ser Ala Gly  
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Gln Val Lys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gln Phe 1n Ile Arg Tyr Gly Leu Ser Gly Lys Glu Val  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ser Thr Ser Tyr Glu Val Gln Val Arg Val Lys Ala Gln Arg Asn  
1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gln Lys Arg Tyr Thr Phe Arg Val Arg Ser Arg Phe Asn Pro Leu  
1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Leu Ser Lys Tyr Asp Val Gln Val Arg Ala Ala Val Ser Ser Met  
1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown



(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Thr Arg Tyr Thr Phe Ala Val Arg Ala Arg Met Ala Pro Ser  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg Leu Asp Gly  
1 5 10 15

International Application No: PCT/

**MICROORGANISMS**

Optional Sheet in connection with the microorganism referred to on page 31, lines 15-35 of the description \*

**A. IDENTIFICATION OF DEPOSIT \***

Further deposits are identified on an additional sheet \*

Name of depositary institution \*

American Type Culture Collection

Address of depositary institution (including postal code and country) \*

12301 Parklawn Drive  
Rockville, MD 20852  
USDate of deposit \* September 14, 1994 Accession Number \* 75885**B. ADDITIONAL INDICATIONS \*** (leave blank if not applicable). This information is continued on a separate attached sheet**C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE \*** (of the indications are not all designated States)**D. SEPARATE FURNISHING OF INDICATIONS \*** (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later \* (Specify the general nature of the indications e.g., "Accession Number of Deposit")

**E.** ☒ This sheet was received with the International application when filed (to be checked by the receiving Office)  
(Authorized Officer)☐ The date of receipt (from the applicant) by the International Bureau \*

was

\_\_\_\_\_  
(Authorized Officer)

Form PCT/RO/134 (January 1981)

International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive  
Rockville, MD 20852  
US

<u>Accession No.</u>	<u>Date of Deposit</u>
75886	September 14, 1994
75887	September 14, 1994
75888	September 14, 1994
75889	September 14, 1994
75890	September 14, 1994
75970	December 14, 1994
75971	December 14, 1994
75972	December 14, 1994
75973	December 14, 1994
75974	December 14, 1994

WHAT IS CLAIMED IS:

1. An isolated nucleotide sequence encoding an Hu-B1.219 protein.

5

2. A cDNA nucleotide sequence encoding an Hu-B1.219 protein.

3. A cDNA nucleotide sequence encoding an Hu-B1.219  
10 protein in which the nucleotide sequence encodes the amino acid sequence of Figure 2A-2G or which is capable of selectively hybridizing to the DNA sequence of Figure 2A-2G.

4. A cDNA nucleotide sequence encoding an Hu-B1.219  
15 protein in which the nucleotide sequence encodes the amino acid sequence of Figure 2A-2G wherein the carboxyl terminal end is replaced by a sequence indicated as Form 2 in Figure 3B, starting at position #7, or which is capable of selectively hybridizing to this DNA sequence.

20

5. A cDNA nucleotide sequence encoding an Hu-B1.219 protein in which the nucleotide sequence encodes the amino acid sequence of Figure 2A-2G wherein the carboxyl terminal end is replaced by a sequence indicated as Form 3 in Figure  
25 3B, starting at position #7, or which is capable of selectively hybridizing to this DNA sequence.

6. A recombinant DNA vector containing a nucleotide sequence that encodes an Hu-B1.219 protein.

30

7. The recombinant DNA vector of Claim 6 in which the Hu-B1.219 nucleotide sequence is operatively associated with a regulatory sequence that controls the Hu-B1.219 gene expression in a host.

35

8. A recombinant DNA vector containing a nucleotide sequence that encodes an Hu-B1.219 fusion protein.

9. The recombinant DNA vector of Claim 8 in which the Hu-B1.219 fusion protein nucleotide sequence is operatively associated with an regulatory sequence that controls the Hu-B1.219 fusion protein gene expression in a host.

5

10. The DNA of Claim 2, 3, 4, 5, 6, 7, 8 or 9 in which the nucleotide sequence is capable of hybridizing under standard conditions, or which would be capable of hybridizing under standard conditions but for the degeneracy of the genetic code to the DNA sequence of Figure 2A-2G.

11. An engineered host cell that contains the recombinant DNA vector of Claim 6, 7, 8 or 9.

15 12. An engineered cell line that contains the recombinant DNA expression vector of Claim 7 and expresses Hu-B1.219.

13. An engineered cell line that contains the recombinant DNA expression vector of Claim 9 and expresses Hu-B1.219 fusion protein.

14. The engineered cell line of Claim 12 which expresses the Hu-B1.219 on the surface of the cell.

25

15. The engineered cell line of Claim 12 which secretes a soluble Hu-B1.219 protein.

16. The engineered cell line of Claim 12 which expresses Hu-B1.219 in the form of ribozyme.

17. The engineered cell line of Claim 12 which expresses a cytoplasmic region of Hu-B1.219.

35 18. The engineered cell line of Claim 13 which expresses the Hu-B1.219 fusion protein on the surface of the cell.

19. The engineered cell line of Claim 13 which secretes a soluble Hu-B1.219 protein.

20. A method for producing recombinant Hu-B1.219,  
5 comprising:

- (a) culturing a host cell transformed with the recombinant DNA expression vector of Claim 6 or 7 and which expresses the Hu-B1.219; and
- (b) recovering the Hu-B1.219 gene product from the cell  
10 culture.

21. A method for producing recombinant Hu-B1.219 fusion protein, comprising:

- (a) culturing a host cell transformed with the  
15 recombinant DNA expression vector of Claim 8 or 9 and which expresses the Hu-B1.219 fusion protein; and
- (b) recovering the Hu-B1.219 fusion protein from the cell culture.

22. An isolated Hu-B1.219 protein.

23. The protein of Claim 22 which is produced by recombinant methods.

24. The protein of Claim 23 having an amino acid sequence as substantially depicted in Figure 2A-2G.

25. The protein of Claim 24 having an amino acid  
30 sequence as substantially depicted in Figure 2A-2G wherein the carboxyl terminal end is replaced by a sequence indicated as Form 2 in Figure 3B, starting at position #7.

26. The protein of Claim 24 having an amino acid  
35 sequence as substantially depicted in Figure 2A-2G wherein the carboxyl terminal end is replaced by a sequence indicated as Form 3 in Figure 3B, starting at position #7.

27. The protein of Claim 22, 23, 24, 25 or 26 which is associated with cell surface plasma membrane.

28. The protein of Claim 22, 23, 24, 25 or 26 which is  
5 secreted.

29. The protein of Claim 22, 23, 24, 25 or 26 in which it is linked to a heterologous protein or peptide sequence.

10 30. An oligonucleotide which encodes an antisense sequence complementary to an Hu-B1.219 nucleotide sequence, and which inhibits translation of the Hu-B1.219 gene in a cell.

15 31. An oligonucleotide which encodes a ribozyme sequence complementary to an Hu-B1.219 nucleotide sequence, and which inhibits translation of the Hu-B1.219 gene in a cell.

20 32. An antibody that binds to Hu-B1.219 protein.

33. The antibody of Claim 32 which is of monoclonal origin.

25 34. The antibody of Claim 32 which competitively inhibits the binding of Hu-B1.219 to a ligand.

35 35. A method for screening and identifying ligands of Hu-B1.219 protein comprising:

- 30 (a) contacting Hu-B1.219 protein with a peptide library such that Hu-B1.219 protein binds to one or more peptide species within the library;
- (b) isolating the Hu-B1.219/peptide combination;  
35 and
- (c) determining the sequence of the peptide.

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1/11

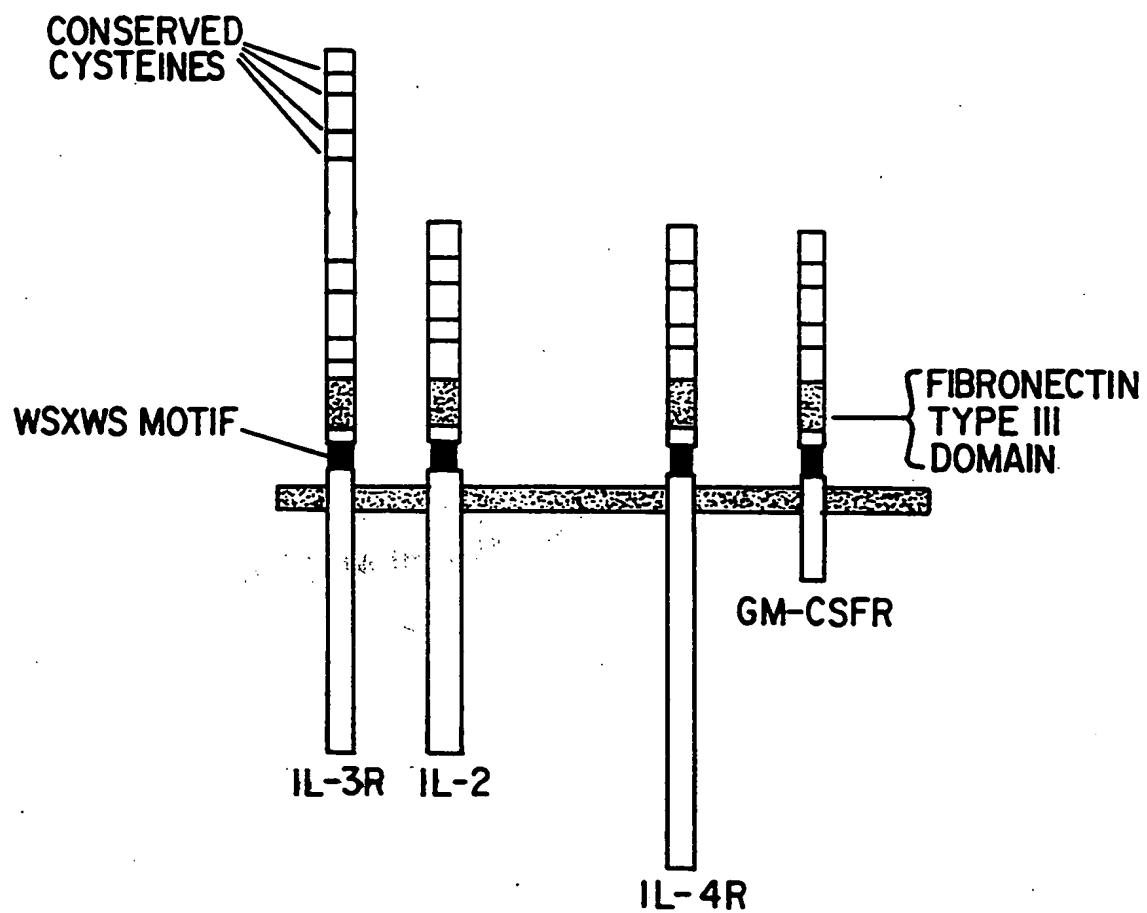


FIG. 1

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314

2/11

9			18			27			36			45			54		
GCG	CGC	GCG	ACG	CAG	GTG	CCC	GAG	CCC	CGG	CCC	GCG	CCC	ATC	TCT	GCC	TTC	GGT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A	R	A	T	Q	V	P	E	P	R	P	A	P	I	S	A	F	G
63			72			81			90			99			108		
CGA	GTT	GGA	CCC	CCG	GAT	CAA	GGT	GTA	CTT	CTC	TGA	AGT	AAG	ATG	ATT	TGT	CAA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
R	V	G	P	P	D	Q	G	V	L	L	*	S	K	M	I	C	Q
117			126			135			144			153			162		
AAA	TTC	TGT	GTG	GTT	TTG	TTA	CAT	TGG	GAA	TTT	ATT	TAT	GTG	ATA	ACT	GCG	TTT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
K	F	C	V	V	L	L	H	W	E	F	I	Y	V	I	T	A	F
171			180			189			198			207			216		
AAC	TTG	TCA	TAT	CCA	ATT	ACT	CCT	TGG	AGA	TTT	AAG	TTG	TCT	TGC	ATG	CCA	CCA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
N	L	S	Y	P	I	T	P	W	R	F	K	L	S	C	M	P	P
225			234			243			252			261			270		
AAT	TCA	ACC	TAT	GAC	TAC	TTC	CTT	TTG	CCT	GCT	GGA	CTC	TCA	AAG	AAT	ACT	TCA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
N	S	T	Y	D	Y	F	L	L	P	A	G	L	S	K	N	T	S
279			288			297			306			315			324		
AAT	TCG	AAT	GGA	CAT	TAT	GAG	ACA	GCT	GTT	GAA	CCT	AAG	TTT	AAT	TCA	AGT	GGT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
N	S	N	G	H	Y	E	T	A	V	E	P	K	F	N	S	S	G
333			342			351			360			369			378		
ACT	CAC	TTT	TCT	AAC	TTA	TCC	AAA	GCA	ACT	TTC	CAC	TGT	TGC	TTT	CGG	AGT	GAG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
T	H	F	S	N	L	S	K	A	T	F	H	C	C	F	R	S	E
387			396			405			414			423			432		
CAA	GAT	AGA	AAC	TGC	TCC	TTA	TGT	GCA	GAC	AAC	ATT	GAA	GGA	AGG	ACA	TTT	GTT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Q	D	R	N	C	S	L	C	A	D	N	I	E	G	R	T	F	V

FIG.2A

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3/11

		441			450			459			468			477			486	
TCA	ACA	GTA	AAT	TCT	TTA	GTT	TTT	CAA	CAA	ATA	GAT	GCA	AAC	TGG	AAC	ATA	CAG	
---		---		---		---		---		---		---		---		---		
S	T	V	N	S	L	V	F	Q	Q	I	D	A	N	W	N	I	Q	
		495			504			513			522			531			540	
TGC	TGG	CTA	AAA	GGA	GAC	TTA	AAA	TTA	TTC	ATC	TGT	TAT	GTG	GAG	TCA	TTA	TTT	
---		---		---		---		---		---		---		---		---		
C	W	L	K	G	D	L	K	L	F	I	C	Y	V	E	S	L	F	
		549			558			567			576			585			594	
AAG	AAT	CTA	TTC	AGG	AAT	TAT	AAC	TAT	AAG	GTC	CAT	CTT	TTA	TAT	GTT	CTG	CCT	
---		---		---		---		---		---		---		---		---		
K	N	L	F	R	N	Y	N	Y	K	V	H	L	L	Y	V	L	P	
		603			612			621			630			639			648	
GAA	GTG	TTA	GAA	GAT	TCA	CCT	CTG	GTT	CCC	CAA	AAA	GGC	AGT	TTT	CAG	ATG	GTT	
---		---		---		---		---		---		---		---		---		
E	V	L	E	D	S	P	L	V	P	Q	K	G	S	F	Q	M	V	
		657			666			675			684			693			702	
CAC	TGC	AAT	TGC	AGT	GTT	CAT	GAA	TGT	TGT	GAA	TGT	CTT	GTG	CCT	GTG	CCA	ACA	
---		---		---		---		---		---		---		---		---		
H	C	N	C	S	V	H	E	C	C	E	C	L	V	P	V	P	T	
		711			720			729			738			747			756	
GCC	AAA	CTC	AAC	GAC	ACT	CTC	CTT	ATG	TGT	TTG	AAA	ATC	ACA	TCT	GGT	GGA	GTA	
---		---		---		---		---		---		---		---		---		
A	K	L	N	D	T	L	L	M	C	L	K	I	T	S	G	G	V	
		765			774			783			792			801			810	
ATT	TTC	CGG	TCA	CCT	CTA	ATG	TCA	GTT	CAG	CCC	ATA	AAT	ATG	GTG	AAG	CCT	GAT	
---		---		---		---		---		---		---		---		---		
I	F	R	S	P	L	M	S	V	Q	P	I	N	M	V	K	P	D	
		819			828			837			846			855			864	
CCA	CCA	TTA	GGT	TTG	CAT	ATG	GAA	ATC	ACA	GAT	GAT	GGT	AAT	TTA	AAG	ATT	TCT	
---		---		---		---		---		---		---		---		---		
P	P	L	G	L	H	M	E	I	T	D	D	G	N	L	K	I	S	

FIG.2B

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FIG. 2.F

4/11

873	882	891	900	909	918
TGG TCC AGC CCA CCA	TTG GTA CCA	TTT CCA CTT	CAA TAT CAA	GTG AAA	TAT TCA
---	---	---	---	---	---
W S S P P	L V P F P	L Q Y Q V	K Y S		
927	936	945	954	963	972
GAG AAT TCT ACA ACA	GTT ATC AGA	GAA GCT GAC	AAG ATT GTC	TCA GCT	ACA TCC
---	---	---	---	---	---
E N S T T	V I R E A	D K I V S	A T S		
981	990	999	1008	1017	1026
CTG CTA GTA GAC AGT	ATA CTT CCT	GGG TCT TCG	TAT GAG GTT	CAG GTG	AGG GGC
---	---	---	---	---	---
L L V D S	I L P G S	S Y E V Q	V R G		
1035	1044	1053	1062	1071	1080
AAG AGA CTG GAT GGC	CCA GGA ATC	TGG AGT GAC	TGG AGT ACT	CCT CGT	GTC TTT
---	---	---	---	---	---
K R L D G	P G I W S	D W S T P	R V F		
1089	1098	1107	1116	1125	1134
ACC ACA CAA GAT GTC	ATA TAC TTT	CCA CCT AAA	ATT CTG ACA	AGT GTT	GGG TCT
---	---	---	---	---	---
T T Q D V	I Y F P P	K I L T S	V G S		
1143	1152	1161	1170	1179	1188
AAT GTT TCT TTT CAC	TGC ATC TAT	AAG AAG GAA	AAC AAG ATT	GTT CCC	TCA AAA
---	---	---	---	---	---
N V S F H	C I Y K K	E N K I V	P S K		
1197	1206	1215	1224	1233	1242
GAG ATT GTT TGG TGG	ATG AAT TTA	GCT GAG AAA	ATT CCT CAA	AGC CAG	TAT GAT
---	---	---	---	---	---
E I V W W	M N L A E	K I P Q S	Q Y D		
1251	1260	1269	1278	1287	1296
GTT GTG AGT GAT CAT	GTT AGC AAA	GTT ACT TTT	TTC AAT CTG	AAT GAA	ACC AAA
---	---	---	---	---	---
V V S D H	V S K V T	F F N L N	E T K		

FIG.2C

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5/11

1305	1314	1323	1332	1341	1350
CCT CGA GGA AAG TTT ACC TAT GAT GCA GTG TAC TGC TGC AAT GAA CAT GAA TGC					
---	---	---	---	---	---
P R G K F T Y D A V Y C C N E H E C					
1359	1368	1377	1386	1395	1404
CAT CAT CGC TAT GCT GAA TTA TAT GTG ATT GAT GTC AAT ATC AAT ATC TCA TGT					
---	---	---	---	---	---
H H R Y A E L Y V I D V N I N I S C					
1413	1422	1431	1440	1449	1458
GAA ACT GAT GGG TAC TTA ACT AAA ATG ACT TGC AGA TGG TCA ACC AGT ACA ATC					
---	---	---	---	---	---
E T D G Y L T K M T C R W S T S T I					
1467	1476	1485	1494	1503	1512
CAG TCA CTT GCG GAA AGC ACT TTG CAA TTG AGG TAT CAT AGG AGC AGC CTT TAC					
---	---	---	---	---	---
Q S L A E S T L Q L R Y H R S S L Y					
1521	1530	1539	1548	1557	1566
TGT TCT GAT ATT CCA TCT ATT CAT CCC ATA TCT GAG CCC AAA GAT TGC TAT TTG					
---	---	---	---	---	---
C S D I P S I H P I S E P K D C Y L					
1575	1584	1593	1602	1611	1620
CAG AGT GAT GGT TTT TAT GAA TGC ATT TTC CAG CCA ATC TTC CTA TTA TCT GGC					
---	---	---	---	---	---
Q S D G F Y E C I F Q P I F L L S G					
1629	1638	1647	1656	1665	1674
TAC ACA ATG TGG ATT AGG ATC AAT CAC TCT CTA GGT TCA CTT GAC TCT CCA CCA					
---	---	---	---	---	---
Y T M W I R I N H S L G S L D S P P					

FIG.2D

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FIG. 1

6/11

1683	1692	1701	1710	1719	1728
ACA TGT GTC CTT	CCT GAT TCT	GTG GTG AAG	CCA CTG CCT	CCA TCC AGT	GTG AAA
---	---	---	---	---	---
T C V L P D S V V K P L P P S S V K					
1737	1746	1755	1764	1773	1782
GCA GAA ATT ACT	ATA AAC ATT	GGA TTA TTG	AAA ATA TCT	TGG GAA AAG	CCA GTC
---	---	---	---	---	---
A E I T I N I G L L K I S W E K P V					
1791	1800	1809	1818	1827	1836
TTT CCA GAG AAT	AAC CTT CAA	TTC CAG ATT	CGC TAT GGT	TTA AGT GGA	AAA GAA
---	---	---	---	---	---
F P E N N L Q F Q I R Y G L S G K E					
1845	1854	1863	1872	1881	1890
GTA CAA TGG AAG	ATG TAT GAG	GTT TAT GAT	GCA AAA TCA	AAA TCT GTC	AGT CTC
---	---	---	---	---	---
V Q W K M Y E V Y D A K S K S V S L					
1899	1908	1917	1926	1935	1944
CCA GTT CCA GAC	TTG TGT GCA	GTC TAT GCT	GTT CAG GTG	CGC TGT AAG	AGG CTA
---	---	---	---	---	---
P V P D L C A V Y A V Q V R C K R L					
1953	1962	1971	1980	1989	1998
GAT GGA CTG GGA	TAT TGG AGT	AAT TGG AGC	AAT CCA GCC	TAC ACA GTT	GTC ATG
---	---	---	---	---	---
D G L G Y W S N W S N P A Y T V V M					
2007	2016	2025	2034	2043	2052
GAT ATA AAA GTT	CCT ATG AGA	GGA CCT GAA	TTT TGG AGA	ATA ATT AAT	GGA GAT
---	---	---	---	---	---
D I K V P M R G P E F W R I I N G D					
2061	2070	2079	2088	2097	2106
ACT ATG AAA AAG	GAG AAA AAT	GTC ACT TTA	CTT TGG AAG	CCC CTG ATG	AAA AAT
---	---	---	---	---	---
T M K K E K N V T L L W K P L M K N					

FIG.2E

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7/11

2169	2178	2187	2196	2205	2214
GGA ACA TGG	TCA GAA GAT	GTG GGA AAT	CAC ACG AAA	TTC ACT TTC	CTG TGG ACA
---	---	---	---	---	---
G T W	S E D	V G N	H T K	F T F	L W T
2223	2232	2241	2250	2259	2268
GAG CAA GCA	CAT ACT GTT	ACG GTT CTG	GCC ATC AAT	TCA ATT GGT	GCT TCT GTT
---	---	---	---	---	---
E Q A	H T V	T V L	A I N	S I G	A S V
2277	2286	2295	2304	2313	2322
GCA AAT TTT	AAT TTA ACC	TTT TCA TGG	CCT ATG AGC	AAA GTA AAT	ATC GTG CAG
---	---	---	---	---	---
A N F	N L T	F S W	P M S	K V N	I V Q
2331	2340	2349	2358	2367	2376
TCA CTC AGT	GCT TAT CCT	TTA AAC AGC	AGT TGT GTG	ATT GTT TCC	TGG ATA CTA
---	---	---	---	---	---
S L S	A Y P	L N S	S C V	I V S	W I L
2385	2394	2403	2412	2421	2430
TCA CCC AGT	GAT TAC AAG	CTA ATG TAT	TTT ATT ATT	GAG TGG AAA	AAT CTT AAT
---	---	---	---	---	---
S P S	D Y K	L M Y	F I I	E W K	N L N
2439	2448	2457	2466	2475	2484
GAA GAT GGT	GAA ATA AAA	TGG CTT AGA	ATC TCT TCA	TCT GTT AAG	AAG TAT TAT
---	---	---	---	---	---
E D G	E I K	W L R	I S S	S V K	K Y Y
2493	2502	2511	2520	2529	2538
ATC CAT GAT	CAT TTT ATC	CCC ATT GAG	AAG TAC CAG	TTC AGT CTT	TAC CCA ATA
---	---	---	---	---	---
I H D	H F I	P I E	K Y Q	F S L	Y P I
2547	2556	2565	2574	2583	2592
TTT ATG GAA	GGA GTG GGA	AAA CCA AAG	ATA ATT AAT	AGT TTC ACT	CAA GAT GAT
---	---	---	---	---	---
F M E	G V G	K P K	I I N	S F T	Q D D

FIG.2F

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PS.015

8/11

2601	2610	2619	2628	2637	2646
ATT GAA AAA CAC	CAG AGT GAT	GCA GGT TTA	TAT GTA ATT	GTG CCA GTA	ATT ATT
-----	-----	-----	-----	-----	-----
I E K H	Q S D A	G L Y V	I V P V	I I	
2655	2664	2673	2682	2691	2700
TCC TCT TCC ATC	TTA TTG CTT	GGA ACA TTA	TTA ATA TCA	CAC CAA AGA	ATG AAA
-----	-----	-----	-----	-----	-----
S S S I	L L L G	T L L I	S H Q R	M K	
2709	2718	2727	2736	2745	2754
AAG CTA TTT TGG	GAA GAT GTT	CCG AAC CCC	AAG AAT TGT	TCC TGG GCA	CAA GGA
-----	-----	-----	-----	-----	-----
K L F W	E D V P	N P K N	C S W A	Q G	
2763	2772	2781	2790	2799	2808
CTT AAT TTT CAG	AAG ATG CTT	GAA GGC AGC	ATG TTC GTT	AAG AGT CAT	CAC CAC
-----	-----	-----	-----	-----	-----
L N F Q	K M L E	G S M F	V K S H	H H	
2817	2826	2835	2844	2853	2862
TCC CTA ATC TCA	AGT ACC CAG	GGA CAC AAA	CAC TGC GGA	AGG CCA CAG	GGT CCT
-----	-----	-----	-----	-----	-----
S L I S	S T Q G	H K H C	G R P Q	G P	
2871	2880	2889	2898	2907	2916
CTG CAT AGG AAA	ACC AGA GAC	CTT TGT TCA	CTT GTT TAT	CTG CTG ACC	CTC CCT
-----	-----	-----	-----	-----	-----
L H R K	T R D L	C S L V	Y L L T	L P	
2925	2934	2943	2952	2961	2970
CCA CTA TTG TCC	TAT GAC CCT	GCC AAA TCC	CCC TCT GTG	AGA AAC ACC	CAA GAA
-----	-----	-----	-----	-----	-----
P L L S	Y D P A	K S P S	V R N T	Q E	
2979	2988				
TGA TCA ATA AAA	AAA AAA AAA 3'				
-----	-----				
* S I K	K K K				

FIG.2G

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S. 618



9/11

			2760	2770	2780	2790	2800	
HuB1.219 Form	1	2751	AGGACTTAAT	TTTCAGAAGA	TGCTTGAAGG	CAGCATGTTC	GTTAAGAGTC	2800
HuB1.219	2	2751	AGGACTTAAT	TTTCAGAAGA	AAATGCCTGG	CACAAAGGAA	CTACTGGGTG	2800
HuB1.219	3	2751	AGGACTTAAT	TTTCAGAAGA	GAACGGACAT	TCTTTGAAGT	CTAATCATGA	2800
			2810	2820	2830	2840	2850	
HuB1.219 Form	1	2801	ATCACCCTC	CCTAATCTCA	AGTACCCAGG	GACACAAACA	CTGCGGAAGG	2850
HuB1.219	2	2801	GAGGTTGGT	GACTTAGGAA	ATGCTTGTGA	AGCTACGTCC	TACCTCGTGC	2850
HuB1.219	3	2801	TCACTACAGA	TGAACCCAAT	GTGCCAACTT	CCCAACAGTC	TATAGAGTAT	2850
			2860	2870	2880	2890	2900	
HuB1.219 Form	1	2851	CCACAGGGTC	CTCTGCATAG	GAAAACCAGA	GACCTTTGTT	CACTTGTTTA	2900
HuB1.219	2	2851	GCACCTGCTC	TCCCTGAGGT	GTGCACAATG	.....	.....	2900
HuB1.219	3	2851	TAGAAGATT	TTACATTCTG	AAGAAGG...	.....	.....	2900
			2910	2920	2930	2940	2950	
HuB1.219 Form	1	2901	TCTGCTGACC	CTCCCTCCAC	TATTGTCCTA	TGACCCTGCC	AAATCCCCCT	2950
HuB1.219	2	2901	.....	.....	.....	.....	.....	2950
HuB1.219	3	2901	.....	.....	.....	.....	.....	2950
			2960	2970	2980	2990	3000	
HuB1.219 Form	1	2951	CTGTGAGAAA	CACCCAAGAA	TGATCAATAA	AAAAAAAAAA	A.....	3000
HuB1.219	2	2951	.....	.....	.....	.....	.....	3000
HuB1.219	3	2951	.....	.....	.....	.....	.....	3000

FIG.3A

			10	20	30	40	50	
HuB1.219 Form	1	1	GLNFQKMLEG	SMFVKSHHHS	LISSTQGHKH	CGRPQGPLHR	KTRDLCSLVY	50
HuB1.219	2	1	GLNFQKKMPG	TKELLGGGWL	T*EMLVKLRP	TSCAPALPEV	CTM.....	50
HuB1.219	3	1	GLNFQKRTDI	L*SLIMITTD	EPNVPTSQOS	IEY*KIFTF*	RR.....	50
			60	70	80	90	100	
HuB1.219 Form	1	51	LLTLPPLLSY	DPAKSPSVRN	TQE*SIKKKK	.....	.....	100
HuB1.219	2	51	.....	.....	.....	.....	.....	100
HuB1.219	3	51	.....	.....	.....	.....	.....	100

FIG.3B

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10/11

SPACING OF CONSERVED AMINO ACIDS IN THE EXTRACELLULAR  
DOMAINS OF KNOWN CYTOKINE RECEPTOR GENES

..C...C.....C.....A/PP.L/V.....W.....Y.....L....Y..R...G.WSXWS..  
(10-11) (28-35) (9-16) (36-48) (2-3) (13-18) (12-20) (26-33) (5-6)(5)(6-11)(2)

CONSERVED AMINO ACIDS IN THE 5' EXTRACELLULAR DOMAINS OF CLONE Hu-B1.219

..C...C.....C.....C.....PP.L.....W.....Y.....L....Y..R...G.WSDWS..  
(11) (44) (10) (43) (3) (14) (12) (30) (5) (5)(8)(2)

CONSERVED AMINO ACIDS IN THE 3' EXTRACELLULAR DOMAINS OF CLONE Hu-B1.219

..C...C.....C.....C.....PP.V.....W.....Y.....P....Y..R...G.WSNWS..  
(11) (41) (10) (41) (3) (15) (16) (27) (6) (5)(8)(2)

FIG.4

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11/11

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mIL2R $\beta$	E P Y L E F E A R R R L L
hIL2R $\gamma$	E H L V Q Y R T D W D H S
mIL5R $\alpha$	D H C F N Y E L K I Y N T
mEPOR	T T H I R Y E V D V S A G
Hu-B1.219(5')	P F P L Q Y Q V K Y Q V K
Hu-B1.219(3')	Q F Q I R Y G L S G K E V

HYDROPHOBIC: "\*"

HYDROPHILIC: "-"

FIG.5

\* b \* b \* b

mIL-2R $\beta$	S T S Y E V Q V R V K A Q R N
hIL-2R $\gamma$	Q K R Y T F R V R S R F N P L
mIL-5R $\alpha$	L S K Y D V Q V R A A V S S M
mEPOR	G T R Y T F A V R A R M A P S
Hu-B1.219(5')	G S S Y E V Q V R G K R L D G
Hu-B1.219(3')	C A V Y A V Q V R C K R L D G
	Y R

HYDROPHOBIC: "\*"

BASIC: "b"

FIG.6

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/10965

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/705, 16/28; C12N 1/21, 5/10, 15/12, 15/62

US CL : 435/6, 69.1, 69.7, 252.3, 320.1; 530/350, 388.22; 536/23.4, 23.5

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 69.7, 252.3, 320.1; 530/350, 388.22; 536/23.4, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Swiss-prot  
searched for amino acid sequence of Figures 2a-2E

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation* of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CELL, Vol. 61, issued 20 April 1990, R. Fukunaga et al., "Expression Cloning of a receptor for Murine Granulocyte Colony-Stimulating Factor", pages 341-350.	1-35
A	TIBS, Vol.15, issued July 1990, D. Cosman et al., "A new cytokine receptor superfamily", pages 265-269.	1-35
A	CELL, Vol. 63, issued 21 December 1990, M. Hibi et al., "Molecular Cloning and Expression of an IL-6 Signal Transducer, gp130", pages 1149-1157.	1-35

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	
*A* document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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*O* document referring to an oral disclosure, use, exhibition or other means	
*I* document published prior to the international filing date but later than the priority date claimed	*G* document member of the same patent family

Date of the actual completion of the international search

06 DECEMBER 1995

Date of mailing of the international search report

11 JAN 1996

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